The Use of Seagrass (*Zostera muelleri*) Habitat by Canada Geese (*Branta canadensis*) in Waikato Estuaries.

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Abstract

Seagrass beds are highly biodiverse habitats delivering key ecosystem functions and services to mankind. *Zostera muelleri* is New Zealand's single seagrass species, and occurs intertidally within several estuaries and sheltered harbours. However, these habitats are globally in decline due to the impacts of multiple stressors including eutrophication, turbidity, coastal urbanisation, sedimentation, and sea level rise. Herbivory by waterfowl is a relatively unknown biotic disturbance that may cause additional stress to these vulnerable seagrass habitats. Canada geese (*Branta canadensis*) were introduced to New Zealand in 1905, and have since been increasing in numbers since a change in species management. In response to increase in Canada geese populations and use of estuaries along the West coast, the Waikato Regional Council commissioned this MSc (Research) study to investigate the consumption of *Zostera* by Canada geese in Kawhia and Whāingaroa (Raglan) harbours, West coast of the North Island, New Zealand.

In order to better understand the grazing pressure placed on seagrass habitats, a three part investigation was conducted. Behaviours of Canada geese on *Zostera* beds were observed in January and February (2019), at two sites in Whāingaroa Harbour, with geese numbers varying between 8 to 200 at any one time. Observations indicated that foraging incorporated a large proportion of their behavioural budget (> 85%), and birds utilised several destructive methods to forage on both above and below-ground *Zostera* biomass. Foraging was significantly reduced by disturbance events less than 30 m away and was also influenced by group size. Repeat observations in June and July 2019, were not possible as geese were no longer present on the *Zostera* beds.

Canada geese samples were collected to investigate bird diet across a temporal scale; from Kawhia between July to November 2019 (n = 33), and from Raglan between August to September 2020 (n = 26). Gut contents analysis showed that more than 70% of specimens consumed solely pasture in the two hours prior to sampling. Bayesian mixing models in MixSIAR were used for $\delta^{15}N$ and $\delta^{13}C$ stable isotope analysis to evaluate the assimilated diet three to four days (plasma), three to four weeks (red blood cells) and several months (primary feathers) prior to sampling. Pasture was the dominant food source (75 to 93%) contributing to all three tissue types.

This study aimed to provide insight into the consumption of seagrass (*Zostera muelleri*) by Canada geese, and determine the proportion of their diet that came from *Zostera* relative to pasture grass. Although Canada geese were observed feeding on *Zostera* during the dry summer months, gut and isotope samples could not support this, as they were collected during the winter/spring months. This difference indicates that the period where Canada geese exploit seagrass was not captured in the isotope study. Post-moult gut and tissue sampling (from late January) would confirm if these birds use the more digestible *Zostera* to meet their nutritional demands during the dry summer season as pasture grass becomes less nutritious or digestible.

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"And whatever you do, whether in word or deed, do it all in the name of the Lord Jesus, giving thanks to God the Father through Him."

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Introduction

1.1. Seagrass

Seagrass beds are ecologically important habitats delivering key ecosystem functions and services including, primary production, carbon sequestration, water quality, and sediment stabilisation (Barnes & Hughes, 1999; Hemminga & Mateo, 1996; Leopardas et al., 2014; Nicastro & Bishop, 2013; Shelton et al., 2017). Their complex structural characteristics also support a large and often diverse faunal community, and provide a nursery habitat for juvenile fish (Barnes & Hughes, 1999; Drylie et al., 2018; Hemminga & Mateo, 1996; Himes-Cornell et al., 2018; Mills & Berkenbusch, 2009; Nicastro & Bishop, 2013; van der Heide et al., 2012). However, seagrass habitats are in a vulnerable condition and have been declining worldwide, due to multiple anthropogenic stressors such as eutrophication, sedimentation, urbanisation, climate change, herbivory, and physical disturbances (Drylie et al., 2018; Himes-Cornell et al., 2018; Macreadie et al., 2014; Matheson & Schwarz, 2007; Shelton et al., 2017; Waycott et al., 2009). The loss of seagrass beds through these disturbances, that often occur simultaneously, has been shown to have large negative impacts on the estuarine systems supported by them (Leopardas et al., 2014; Nicastro & Bishop, 2013; Waycott et al., 2009). The annual global decline of seagrass has been estimated between 2 to 5%, with a 29% loss of overall seagrass over the last 20 to 50 years (Shelton et al., 2017; Waycott et al., 2009).

1.1.1. Seagrass trends and studies within New Zealand

Zostera muelleri (Zosteraceae) is New Zealand's single seagrass species, (Graeme, 2012a; Graeme, 2012b; Hailes, 2006) and exists almost exclusively in the mid to low intertidal zone (Inglis, 2003; Schwarz, 2004; Turner & Schwarz, 2006b). This species typically inhabits estuaries and sheltered harbours with mud to sand substrates (Inglis, 2003; Turner & Schwarz, 2006b). *Z. muelleri* (herein referred to as *Zostera*) is a marine angiosperm that may produce seeds from October to June, peaking in late summer (Ramage et al., 1998; Santos et al., 2017). The growth rates of *Zostera* leaves and roots/rhizomes are typically highest during spring and summer (Schwarz, 2004; Turner & Schwarz, 2006a) when plants utilise the increase in light availability to maximise their photosynthetic rate, even during low tide emersion (Drylie et al., 2018; Hemminga & Mateo, 1996; Schwarz, 2004). However, disturbances to *Zostera* beds are likely to inhibit growth and resilience of this species in New Zealand (Santos & Matheson,

2017). Similar to worldwide trends, most New Zealand harbours display a decline in *Zostera* due to continuous anthropogenic disturbances (Himes-Cornell et al., 2018; Matheson & Schwarz, 2007; Santos et al., 2013; Turner, 2007; Turner & Schwarz, 2006a; Zabarte-Maeztu et al., 2020). Although there are some exceptions that have seen an overall increase in *Zostera* biomass in the past few decades; Whangapoua Harbour, Wharekawa Harbour, Whangamata Harbour (Turner, 2007), Waitematā Harbour (Meola Reef) (Parkes & Lundquist, 2018), and Whangarei Harbour (Inglis, 2003; Matheson et al., 2017).

Within New Zealand, studies have observed the effect of sedimentation disturbance on *Zostera* growth (Matheson & Schwarz, 2007), presence, abundance and health (Zabarte-Maeztu et al., 2020). Previous studies also include the effects of water column turbidity on primary production of *Zostera* (Drylie et al., 2018), and the reproductive output of *Zostera* based on shore position (Ramage & Schiel, 1998). Herbivory of *Zostera* by waterfowl is a biotic disturbance that has previously been observed by black swans in Tauranga Harbour (Santos et al., 2012), and Golden Bay (Dixon, 2009). Black swans differ in their grazing behaviour relative to Canada geese, typically feeding when *Zostera* is submerged (Santos et al., 2012), whereas Northern Hemisphere studies suggest Canada geese (Northern Hemisphere) tend to feed at low tide or in shallow water (Buchsbaum & Valiela, 1987; Ganter, 2000; Rivers & Short, 2007). To the best of my knowledge there has been only one previous study conducted in New Zealand on the grazing of Canada geese, which was focussed on pasture consumption (Win, 2001). This thesis is therefore the first study to investigate the consumption of *Zostera muelleri* by Canada geese (*Branta canadensis*) in New Zealand.

1.1.2. Concern for seagrass in Kawhia and Whāingaroa harbours

Kawhia and Whāingaroa harbours are two West coast estuaries in the North Island of New Zealand. Due to the presence of *Zostera muelleri*, and the support this species provides to ecosystem structure and function in these systems, it is important to identify and investigate disturbances that have the potential to affect the state of these vulnerable habitats (Graeme, 2012a; Graeme, 2012b). Environmental disturbances likely to affect estuarine vegetation in these harbours include land run-off (sedimentation), uncontrolled stock access, wild animals, and weeds (Graeme, 2012a; Graeme, 2012b). Historical data on *Zostera* beds within New Zealand is limited and not well documented (Turner & Schwarz, 2006b), with little information for West coast harbours in particular. The Waikato Regional Council has monitored and documented the presence of *Zostera* in Kawhia since 2005, and in Raglan since 2007.

Currently, the largest beds in Kawhia are located throughout the mid-harbour flats with minimal disturbances (Graeme, 2012a), while in Whāingaroa Harbour *Zostera* is present alongside the busy town foreshore and up the Opoturu river arm (Graeme, 2012b). These *Zostera* beds may provide an attractive food source to Canada geese, as they are easily accessible, and often more digestible than terrestrial pasture during the dry summer season (Balsby et al., 2017; Ganter, 2000; Inger et al., 2006b). Given that seagrass has been shown to contribute to the diet of these birds overseas (Buchsbaum & Valiela, 1987; Rivers & Short, 2007), consumption of *Zostera* by Canada geese in New Zealand may pose both direct (foraging/consumption) and indirect (defaecation) threats to the conservation of this seagrass species. Therefore, insight into the proportion of Canada goose diet comprised of seagrass, will provide some understanding of the pressures these birds may place on this important habitat-forming species.

1.2. Canada geese

Previous studies on Canada geese (*Branta canadensis*) have predominantly been conducted in the Northern Hemisphere (Buchsbaum & Valiela, 1987; Buchsbaum et al., 1986; Cadieux et al., 2005; Eaton et al., 2017; Foley & Glaser, 2015; Sedinger & Raveling, 1988; Washburn & Seamans, 2012), with limited studies and documentation on population numbers of Canada geese in New Zealand, mainly from the South Island (Coleman, 2008; Smith, 2019; Spurr & Coleman, 2005; White, 1986; Win, 2001). Unlike Canada geese in the Northern Hemisphere, geese in New Zealand are not migratory (White, 1986), which may lead to differences in general behaviour. As such, understanding the behaviour and dietary patterns in New Zealand coastal regions, is a critical first step towards furthering available information of this species.

1.2.1. Introduction and legislation in New Zealand

Fifty *Branta canadensis* (herein referred to by their common name), were introduced into New Zealand from the U.S.A in 1905, as a recreational hunting resource by Fish & Game New Zealand (Imber, 1971; Spurr & Coleman, 2005; Win, 2001). Initially Canada geese were under complete protection to allow population numbers to increase, and in 1931 this was removed to allow hunting (except in specific coastal areas) by waterfowl hunting license holders only (White, 1986). In 1953, the Wildlife Act was introduced by the New Zealand parliament to outline the protection and control of wild animals and birds, and the management of game species (Wildlife Act, 1953). Canada geese were placed under the fourth schedule in the

Wildlife Act, classing them as an unprotected species. This allowed geese to be hunted and killed by all parties concerned, by any means deemed necessary (Spurr & Coleman, 2005; Wildlife Act, 1953). In 1959, management of the species was taken over by the Department of Conservation (DoC) and transferred to the third schedule where geese could only be hunted or killed subject to the Minister's notification (White, 1986). Due to several incidents involving aircraft and harassment of the native paradise shelduck (*Tadorna variegata*), by hunters, Canada geese were transferred to the first schedule of the Wildlife Act in 1973. Being classed as a game bird enabled control of the hunters, as only license holders were able to shoot them (White, 1986).

Due to a continuous increase in numbers, Canada geese were changed to schedule five of the Wildlife Act in 2011 (wildlife not protected). Management of the species was no longer conducted by DoC or Fish & Game, but there was hope that population numbers would be controlled by a proactive response from hunters, farmers, and landowners. However, due to a lack of coordination between these parties, a reduction in Canada goose numbers has not yet been achieved (Spurr & Coleman, 2005), in fact populations appear to be increasing. In 2013, there was estimated to be more than 60,000 Canada geese across New Zealand, and in 2018, more than 10,000 geese were counted across the Waikato region (Smith, 2019). This rise in Canada goose numbers has led to a rise in complaints from farmers and private landowners about their presence. Their concerns are primarily regarding the consumption of their pasture and crops (Smith, 2019; Spurr & Coleman, 2005), as well as bird fouling deterring their livestock from foraging on areas of their land (Spurr & Coleman, 2005). As such, also understanding the importance of pasture grass as a food source, provides insight into some of the likely impacts of Canada geese higher up in the catchment and the potential for downstream effects (e.g., increased sedimentation and nutrient input).

1.2.2. General behaviour

In New Zealand, Canada geese are residential, rather than migratory birds (White, 1986) and are generally found on pasture near lakes, rivers, and estuaries (Smith, 2019). They display strong social bonds (Raveling, 1979) and tend to move around in family flocks (White, 1986) which increases detectability of potential food sources and threats (e.g., human disturbance) (White, 1986). Individual flocks may select different feeding sites but reunite at the same roosting sites each evening (White, 1986). They typically fly between their roost and feeding sites at sunrise and sunset (White, 1986). However, during times of heightened hunting

pressures, geese may select to feed during the night (White, 1986). This demonstrates the highly adaptive nature of Canada geese, by using a diverse range of habitats to meet their behavioural and nutritional requirements (White, 1986; Win, 2001).

Previous studies indicate that these geese predominantly feed on grasses (high fibre content), young shoots and aquatic vegetation (low fibre content) (Smith, 2019; White, 1986; Win, 2001). Diet selection may change throughout the year dependent on accessibility, food quality and quantity available (White, 1986). According to a South Island study, Canada geese move towards the coast in the summer following their moult but feed on surrounding pasture grass (Spurr & Coleman, 2005). However, grazing behaviour may differ between the North and South Island populations of Canada geese, as there have been numerous sightings from the local community in Waikato estuaries (North Island), of Canada geese foraging on the *Zostera* beds during this dry summer season, while the tide is out.

1.2.3. Canada geese breeding and moult periods

The breeding season for Canada geese in New Zealand begins in late September or early October with the laying of 2 to 10 eggs that require incubation for 26 to 28 days until hatching (Spurr & Coleman, 2005; White, 1986; Win, 2001). Hatching is timed to make the most of the spring vegetation growth (Raveling, 1979) to enable goslings to increase their body mass by more than 20 times in under two months (Cadieux et al., 2005). Goslings become fully fledged and ready for flight by mid-January/early February (Spurr & Coleman, 2005; White, 1986; Win, 2001). This timing is synchronised with the late December/early January moult and primary flight feather regrowth of adult geese (Raveling, 1979; Spurr & Coleman, 2005; White, 1986). Geese then often leave their inland breeding areas and gather on coastal lakes and estuaries, likely in response to dryer or drought conditions (Choney et al., 2014; Spurr & Coleman, 2005; Win, 2001), before returning to inland areas for the winter period (Spurr & Coleman, 2005).

Regrowth of moulted primary flight feathers is a critical period for Canada geese as the process is energetically costly (Lewis et al., 2010; Murphy, 1996), and being flightless leaves them vulnerable to predation (Fox et al., 2009; White, 1986). In New Zealand, there are no natural predators or survival risks for this species other than hunting pressures by humans (White, 1986). Readily digestible food sources with high nutrient content are utilised to meet the high energetic costs for feather regrowth, which are grown within 30 to 35 days (Fox et al., 2009;

Raveling, 1979; Spurr & Coleman, 2005; White, 1986). The remaining body and down feathers are not fully regrown until late autumn/start of winter (White, 1986).

1.3. Stable isotopes

Stable isotope analysis is a valuable tool used in foraging ecology studies to determine the proportionate contributions of two or more isotopically distinct food sources to a consumer's diet (Hobson & Clark, 1992b; Inger et al., 2006a; Inger et al., 2006b; Jardine et al., 2003; Parnell et al., 2013) and can provide understanding of comparatively long-term dietary information (Hobson & Clark, 1992a). This method has previously been used on blood and feather tissues in key avian studies on captive Japanese quail (*Coturnix japonica*) (Hobson et al., 1993; Hobson & Clark, 1992a; Hobson & Clark, 1992b), captive Dunlin (*Calidris alpina*) (Ogden et al., 2004), and captive Garden Warblers (*Sylvia borin*) (Hobson & Bairlein, 2003). Previous studies on herbivorous waterfowl include Bewick's swans (*Cygnus columbianus bewickii*), European Mallards (*Anas platyrhynchos*) (Hahn et al., 2012), and Brent geese (*Branta bernicla*) (Inger et al., 2006a; Inger et al., 2006b); the latter being closely related to our species of interest (*Branta canadensis*). However, the extent of isotopic studies conducted on wild avian species remains limited (Inger et al., 2006a; Inger et al., 2006b).

1.3.1. Determination of isotope fractionation from source to tissue

Fractionation is the separating process of lighter and heavier isotopes as they are incorporated into an organism's tissues by selective biochemical assimilation (Jardine et al., 2003; Unkovich et al., 2001), and is different among species, diets and tissue types (Hobson & Clark, 1992b; Vanderklift & Ponsard, 2003). Therefore, based on the fractionation that occurs between the food source and the tissue of interest, species-specific fractionation values are appointed to individual tissues. These are known as trophic discrimination factors (TDF) (Alisauskas & Hobson, 1993; Bond & Diamond, 2011; Caut et al., 2009; Cherel et al., 2005; Hobson et al., 1993; Hobson & Bairlein, 2003; Hobson & Clark, 1992a; Hobson & Clark, 1992b).

Most studies agree that fractionation rates for both $\delta^{13}C$ and $\delta^{15}N$ isotopes differ among tissue types, food sources and species (Alisauskas & Hobson, 1993; Hahn et al., 2012; Hobson & Clark, 1992b; McCutchan et al., 2003; Unkovich et al., 2001). By applying appropriate TDF, multiple tissue types of an individual species can be examined to provide dietary information across a temporal scale (Grecian et al., 2015; Hobson et al., 1993; Hobson & Clark, 1992a;

Inger et al., 2006a; Quillfeldt et al., 2008; Unkovich et al., 2001) although, some captive studies choose to repeatedly sample one tissue type (e.g., claws, blood) on live specimens through time (Alisauskas & Hobson, 1993; Bearhop et al., 2003). Though not always plausible, sampling multiple tissues from the same individual is advantageous to isotope studies to account for isotopic variation that may occur between its own tissues due to differing metabolic processes (Grecian et al., 2015; Unkovich et al., 2001).

An assumption has been made in some previous studies sampling multiple tissue types (e.g., red blood cells and plasma), that the influence of tissue-specific fractionation is minimal in comparison to the dietary change they aim to measure (Inger et al., 2006a; Inger et al., 2006b; Quillfeldt et al., 2008). This assumption is likely used because collecting species and tissue specific fractionation data can be difficult and time consuming, involving long-term (> year) controlled feeding experiments. Due to this, there is a lack of data for avian species and choosing appropriate TDF is often based on the next best available fractionation values (i.e., from as closely related a species as possible), to reduce uncertainty and unexplained variation in estimated dietary proportions (Grecian et al., 2015; Hahn et al., 2012; Parnell et al., 2013; Unkovich et al., 2001) However, the reliability of this approach depends on the understanding of the various processes involved in assimilation of food sources to different tissues, which are inherently complex (Hobson et al., 1993).

1.3.2. Differences present among avian tissues

Blood and feather tissue samples are most commonly used in avian studies to examine an organism's diet across a temporal scale (Alisauskas & Hobson, 1993; Bearhop et al., 2003; Bearhop et al., 2002; Hahn et al., 2012; Hobson & Clark, 1992a; Hobson & Clark, 1992b; Peterson & Fry, 1987; Quillfeldt et al., 2008). Blood is a fast metabolising tissue, so reflects the recent diet of the organism (Alisauskas & Hobson, 1993; Bearhop et al., 2003; Bearhop et al., 2002; Hobson & Clark, 1992a; Unkovich et al., 2001) and can be separated by centrifugation into plasma and red blood cells (RBC). Plasma has a rapid turnover rate of three to four days, whereas the turnover rate of RBC is three to four weeks (Hahn et al., 2012; Hong et al., 2019; Inger et al., 2006a) enabling comparison of diet over these time frames. Separating whole blood into plasma and RBC is also beneficial in that the potential negative influence of isotopically depleted lipids and uric acid can be removed from the RBC samples (Peterson & Fry, 1987; Quillfeldt et al., 2008). Without separation, lipids (depleted in $\delta^{13}C$), and uric acid

(depleted in $\delta^{15}N$), may cause blood samples to show lower isotopic results than actually assimilated from the diet (Bearhop et al., 2000b; Zanden & Rasmussen, 2001). However, the presence of lipids and uric acid in avian blood are often minimal (< 1%) and so are unlikely to affect isotopic results and comparisons between tissues (Bearhop et al., 2000a; Hong et al., 2019; Zanden & Rasmussen, 2001).

Feathers are an isotopically inert tissue, so reflect the diet at the time the feathers were grown (see section 1.1.3). Feathers carry the fixed isotopic composition of their diet during synthesis following their annual moult (Alisauskas & Hobson, 1993; Bearhop et al., 2003; Caccamise et al., 2000; Fox et al., 2009; Grecian et al., 2015; Hahn et al., 2012; Hobson & Clark, 1992a; Hong et al., 2019; Munafo & Gibbs, 2012; Paritte & Kelly, 2009; Unkovich et al., 2001; Viljoen et al., 2016). Therefore, knowledge on a species' moult timing is essential to reliably determine their diet in reference to a past point in time (Bearhop et al., 2003; Fox et al., 2009; Grecian et al., 2015).

1.3.3. The use of anticoagulants in blood studies

Anticoagulants are often used to prevent blood from clotting and enable centrifugation to separate the plasma and RBC at a later point in time (Booth & Wright, 2016; Ehret et al., 2002; Hoye, 2012; Washburn & Seamans, 2012). Lithium/Sodium Heparin and Ethylenediaminetetraacetic acid (EDTA) are commonly used anticoagulants that coat the inside of vacutainers used for collecting blood (Ehret et al., 2002; Osorio, 2010). Limited information on the use of these two anticoagulants for isotope studies exists, particularly in avian studies where no published literature on their use for herbivorous waterfowl sampling was found. Furthermore, there is seemingly no specific rationale for using one anticoagulant over another, except for PCR purposes (Ehret et al., 2002). A previous study on Green sea turtles (*Chelonia mydas*) found that plasma and RBC samples with EDTA treatment were $\delta^{15}N$ depleted, with whole blood being nitrogen enriched using sodium heparin (Lemons et al., 2012). However, there are more studies that have found no significant $\delta^{13}C$ and $\delta^{15}N$ differences using heparin or EDTA on blood samples from sharks (Weideli et al., 2019), humans (Osorio, 2010), and chickens (Denadai et al., 2019). The only avian study found, examining chicken blood showed no influence of either EDTA or heparin anticoagulants to isotope values in comparison to control samples (no anticoagulant) (Denadai et al., 2019).

1.3.4. Incorporation of potential food sources

Stable isotope analyses of $\delta^{13}C$ and $\delta^{15}N$ can be a useful tool for understanding the proportion of each dietary food source assimilated into the tissues of the study organism and how this potentially changes over time (Bearhop et al., 2002; Hobson & Clark, 1992a; Hobson & Clark, 1992b; Inger et al., 2006a; Peterson & Fry, 1987). Their assimilated diet represents the composition of isotopic signatures that have fractionated from the food source to the tissue, rather than just the bulk ingested diet (Zanden & Rasmussen, 2001). An organism's foraging preferences and the contributions of each food source to tissue formation is reflected by the isotopic composition of each individual tissue (Alisauskas & Hobson, 1993; Bearhop et al., 2002; Caccamise et al., 2000; Grecian et al., 2015; Hobson & Clark, 1992a; Hobson & Clark, 1992b; Quillfeldt et al., 2008). Within this study, food sources considered to potentially contribute to the diet of Canada geese included pasture grass, seagrass (*Zostera*), and various macroalgae species. Due to differing photosynthetic pathways, seagrass and macroalgae are classed as C_4 marine plants and pasture grass species are predominantly classed as C_3 terrestrial plants (Alisauskas & Hobson, 1993; Hemminga & Mateo, 1996; Hobson & Clark, 1992a; Inger et al., 2006a; Jardine et al., 2003; Tieszen et al., 1983). Consequently, the isotopic signatures of these food sources are easily distinguishable even if geese frequently travel between the two habitats (Fox et al., 2009; Hahn et al., 2012).

Juvenile Yellow-bellied flounder (*Rhombosolea leporina*) were also considered a potential food source for Canada geese as local Iwi and hunters indicated that waterfowl are often found with near-intact specimens in their stomachs. Indeed, reports of a swan from Kawhia Harbour containing more than 80 juvenile flounder made local and national news at the time this current study began (Calder, 2018; Smith, 2019). The isotopic signature of flounder would be expected to sit at a higher trophic level, represented by a greater $\delta^{15}N$ value, than vegetation sources.

1.4. Study objectives

Although there are growing concerns for the conservation of seagrass habitats in New Zealand and globally (Turner & Schwarz, 2006b; Waycott et al., 2009), there have been very few foraging ecology studies investigating the use of this habitat by herbivorous waterfowl (Balsby et al., 2017; Clausen et al., 2012; Ganter, 2000; Santos et al., 2012; Zipperle et al., 2010). Given the proliferation of Canada geese in New Zealand since the most recent change in legislation

(2011), there is a need to better understand how these organisms utilise and potentially impact upon the systems in which they reside.

This MSc (Research) project was funded by the Waikato Regional Council in response to growing concerns from West coast communities regarding the perceived increase in Canada geese populations in the estuaries and on the surrounding farmland. The key aim of this study was to provide insight into the utility of *Zostera* patches by Canada geese and determine the proportion of their diet that comes from these fragile, yet functionally important ecosystems. The first objective was to conduct field observations, to determine bird behaviour during their time spent on *Zostera* beds and to document the mechanisms by which plants are disturbed during grazing. The second objective was to collect tissue and gut content samples to assess if the most recently ingested material, reflected their assimilated diet. Assimilated food sources provide insight into their diet over time and the relative importance of *Zostera* as a food source used in tissue formation.

My key goals were therefore:

- To determine how much time Canada geese (*Branta canadensis*) spend foraging while on seagrass (*Zostera muelleri)* beds, and whether behaviour changed in response to the proximity of different disturbances or different group sizes.

- To identify how much of the most recently ingested food by *B. canadensis* is comprised of *Zostera*, and whether this is dependent on bird size or sex.

- To evaluate the proportion of *Zostera* assimilated into *B. canadensis* tissues, relative to other food sources, and whether a temporal shift in diet selection (days to months) could be detected through isotopic analysis of three different tissue types (plasma, red blood cells, and primary feathers).

Chapter Two

Methods

2.1. Study area

Canada geese samples were collected from two harbours, Whāingaroa (Raglan) and Kawhia, West coast of the North Island, New Zealand (Figure 1). Behavioural observations of Canada geese were conducted at two site locations within the Whāingaroa Harbour. Cliff street (Site A: 37°47.891'S, 174°52.062'E) and the Opoturu river arm (Site B: 37°48.675'S, 174°52.062'E) were selected due to the presence of geese on seagrass beds and the ability to make observations without disturbance (Figure 2). Behavioural observations were not conducted in Kawhia Harbour because there were no accessible observation points in proximity to the seagrass beds where birds were frequently observed.

Figure 1. Study locations of Whāingaroa and Kawhia harbours within Waikato, West coast of the North Island, New Zealand.

Figure 2. Study locations used for behavioural observations in Whāingaroa, West coast of the North Island, New Zealand.

2.2. Behavioural analysis

2.2.1. Behavioural determination

An ethogram was formulated by referring to listed behaviours from previous studies (Foley & Glaser, 2015; Paulus, 1988; Sedinger & Raveling, 1988). Observations of Canada geese were trialled at Hamilton lake, in order to become familiar with their behaviours and refine methodology. The ethogram (Table 1) provided a fixed definition for the behaviours viewed on the seagrass beds in Whāingaroa Harbour.

Table 1. Definitions for the observed behaviours of Canada geese (*Branta canadensis*).

Behaviour	Definition
Foraging	Head is reaching down while standing still, walking, or swimming to grab at the food source with its beak.
Vigilance	Head and neck are stretched upwards to scan the surroundings while standing still or walking.
Walking	Head is upwards (but not stretched), and the goose is moving from one location to another by placing one foot in front of the other.
Loafing	The goose is moving its beak through its feathers or has its head tucked under its wing, while either sitting or standing on one or both legs.
Swimming	Head is upwards (but not stretched), and the goose is moving through the water from one location to another without its feet touching the ground.

2.2.2. Sampling design

Preliminary surveillance of the seagrass beds from sunrise to sunset and at high and low tides, established the time of day and tide when geese would most likely be present. Following this, behavioural observations were conducted starting at sunrise (~ 6:30 AM) during low tide and continued until the *Zostera* bed was covered with water and/or the geese had left the site (approximately two to four hours). If geese from observation site A left before the seagrass inundation, observations were continued at site B if birds were present. Observations were carried out from a discrete vantage point overlooking >1000 m^2 of the intertidal flat. Date, time of day, and general weather conditions were recorded on each occasion.

A Nikon Coolpix P1000 Digital Camera and Bak4 7x50 waterproof binoculars were used to take relevant photos and study behaviours from a discreet distance. An initial headcount of all

the geese present on the seagrass bed was performed before observations began. Then at a fixed interval of five minutes, the number of geese performing each behaviour was recorded (Sedinger & Raveling, 1988). If necessary, a headcount was also conducted to ensure that the total number of geese being observed had not changed since the previous recording. Different potential disturbances such as people walking-by, watercraft, dogs, and loud noises were recorded and their proximity to the birds, at the time that they occurred, was noted over the course of each observation period. Observations continued for up to four hours or until the geese had left the area, whichever occurred first. Geese were monitored for a total of 28 hours and 45 minutes, across nine days in January and February 2020 (within the austral summer period). These observations were attempted again in June 2020; however, no birds were recorded on the seagrass beds at this time.

2.3. Sample collection

In order to sample tissues from Canada geese, a network of hunters and farm/land owners was established through Waikato Fish and Game, local online community boards, discussions with local iwi (Kawhia), and by directly contacting individual landowners. Samples were collected during the austral winter and spring seasons, from multiple farms surrounding the Raglan and Kawhia harbours to get a spread of the goose population. Geese from Kawhia (n = 33) were collected sporadically between July to November 2019. Black swans (n = 11) were also collected from Kawhia in this sampling period to enable comparisons of the diet between the two species, as black swans are known seagrass consumers (Santos et al., 2012; Santos et al., 2013). The black swans were processed following the same procedures as Canada geese, detailed below. Geese from Raglan (n = 26) were shot in August to September 2020. The number of geese collected each time was dependent on the success of the hunters. An attempt was made to get post-moult samples from Canada geese, but only four geese from Kawhia were collected before Covid-19 movement restrictions stopped all further sampling. Gut contents and feather samples were collected from all four geese, but only two of the geese had an adequate amount of extractable blood for separation into plasma and red blood cells. Therefore, these birds were excluded from further analysis.

2.3.1. Blood tissue

Blood samples were collected from the geese within eight hours of death (Booth & Wright, 2016; Viljoen et al., 2016), using a 23-gauge needle on a 10 ml syringe. After drawing 4 to 8

ml of blood from the heart and nearby arteries, the sample was immediately transferred to a labelled 9 ml Ethylenediaminetetraacetic acid (EDTA) vacutainer and gently inverted three times to ensure adequate contact with the anticoagulant. Duplicate samples were drawn in most instances. A literature review of previous studies found no significant differences in isotopic ratios between blood collected from sharks, sea turtles, and poultry in EDTA or Sodium Heparin (SH) vacutainers (Denadai et al., 2019; Lemons et al., 2012; Osorio, 2010; Weideli et al., 2019). However, a decision was made to use EDTA as comparisons between the two anticoagulants as preliminary analysis revealed minor enrichment of $\delta^{15}N$ in the SH in Canada geese samples (Appendix Table 2. 1 & 2).

Blood samples were centrifuged at a speed of 2200 x G for 20 minutes (Ehret et al., 2002) at ambient room temperature within three hours of the blood being drawn. Centrifuged samples (Figure 3A) were then kept in a refrigerator (4°C) until the separated plasma and red blood cells (RBC) could be extracted into individual pottles (usually overnight). Separation was done using a 1 ml auto-pipette for the plasma and a 1 ml standard pipette for the RBC. The thin layer of fat between the plasma and RBC was removed into a waste container. Samples were then frozen at − 20°C for at least 24 hours before freeze-drying. Freeze-dried blood samples were ground using a small mortar and pestle (Figure 3B) and stored in pottles, until isotope analysis.

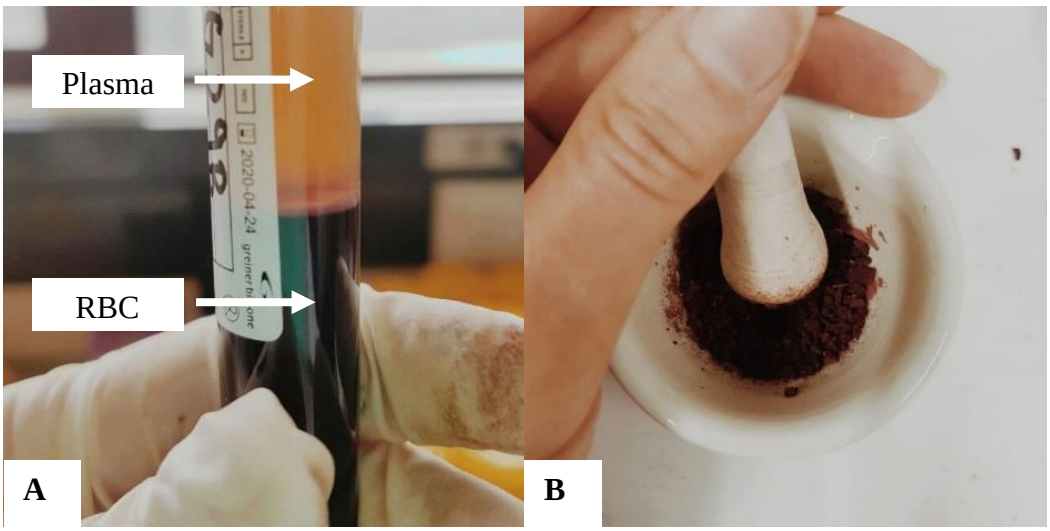

Figure 3. Centrifuged blood sample in an EDTA vacutainer (A); ground freeze dried red blood cells (RBC) in a mortar and pestle (B).

2.3.2. Feather tissue

The first three primary feathers (Paritte & Kelly, 2009) from both wings were clipped approximately at a length of 15 cm. Feathers were washed in warm water with detergent to remove dried blood, oils, and grime before gently rinsing in IPA 70% to remove remaining lipids and lyse microorganisms (Paritte & Kelly, 2009). They were then rinsed once more in warm water and hung to completely airdry before the required sample size was cut. Approximately four to five cm from the tip of the vane (excluding the rachis) was cut for all six primary feathers and amalgamated into one sample for each goose following the protocols of Grecian et al., 2015 (Figure 4). The feather samples were placed into aluminium foil plates and dried in a contherm oven at 40°C for 24 hours to remove any remaining moisture. Following this, the feathers were cut into fine segments using scissors (to the same effect as being ground) and stored in sealed envelopes (Viljoen et al., 2016).

Figure 4. Feather preparation for isotope analysis. Approximately four to five cm of the tip of the primary feather vane was cut and placed into an aluminium foil plate.

2.3.3. Gut content

The proventriculus and gizzard was removed from each goose by attaching a clamp at the beginning of the proventriculus before cutting with surgical scissors. Remaining sinews holding the gizzard in place and the duodenum entrance were cut to allow complete detachment of the stomach. A large incision was made up the proventriculus and through the two sides of the gizzard (Figure 5) to open it out. This enabled all the stomach contents to be removed and rinsed into a 1 L pottle with 70% IPA.

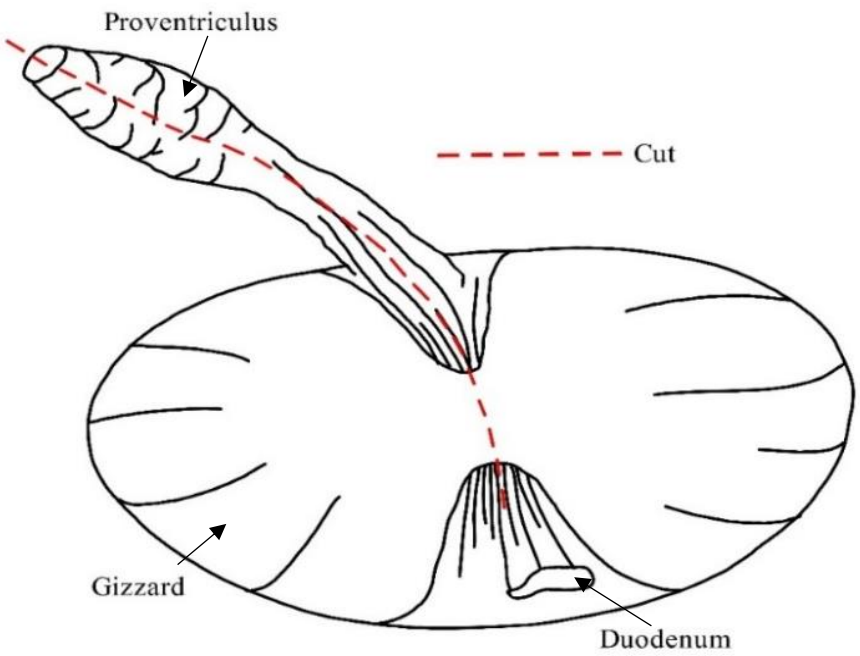

Figure 5. Schematic diagram of a digestive tract of the Canada goose (*Branta canadensis*). The red line indicates the incision made to remove the stomach contents from each of the birds sampled (n = 59).

2.3.4. Metadata

Length, wingspan, and sex metadata were recorded for each goose to provide further information on population dynamics and to aid in identifying any patterns within the results. Sex was determined by identifying their reproductive organs (Figure 6). The wingspan was measured from tip-to-tip of both outstretched wings and length was measured from the beak tip to the tip of the feet (Figure 7).

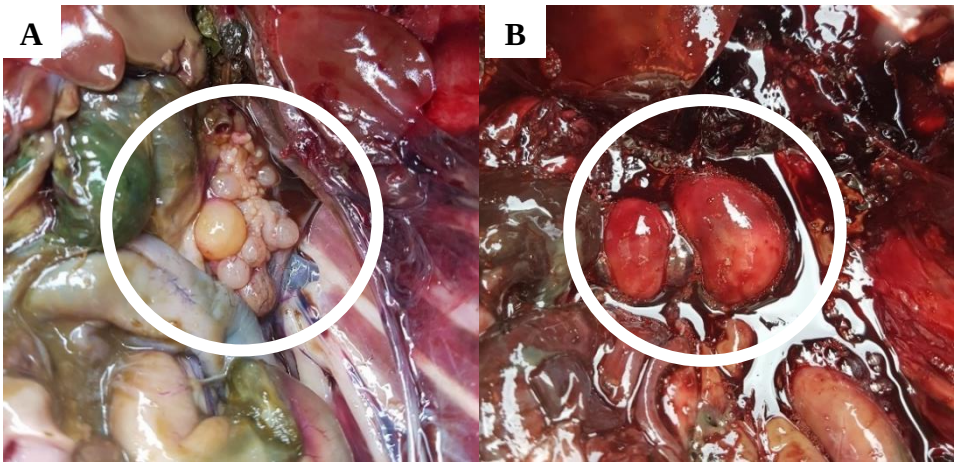

Figure 6. Determination of the sex of Canada goose (*Branta canadensis*). Ovaries of an adult female (A) and testes of an adult male (B).

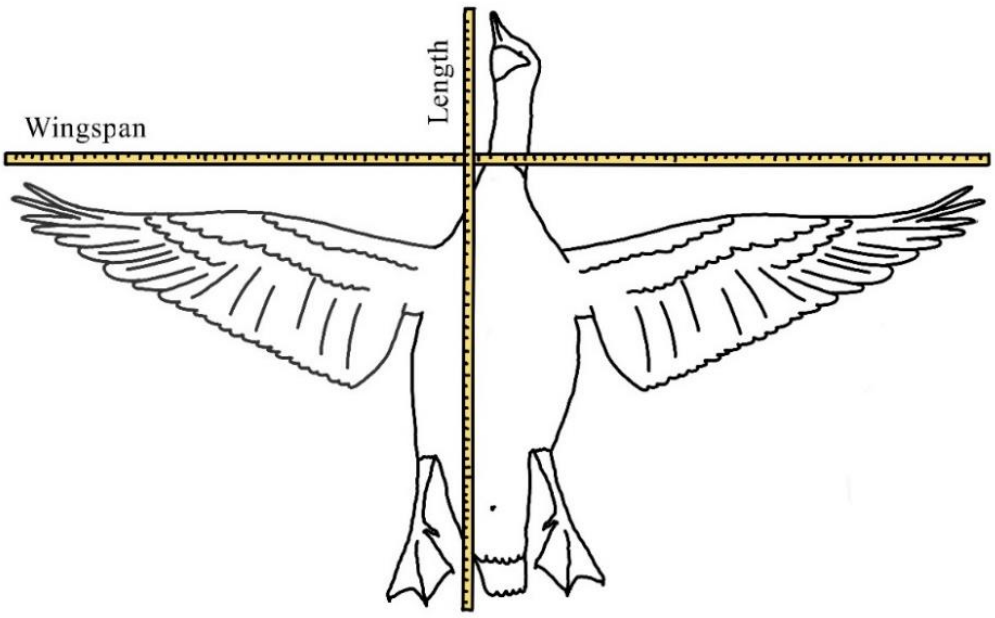

Figure 7. Schematic diagram of a Canada goose being measured from beak to toe (length) and wing tip to wing tip (wingspan).

2.3.5. Food sources

Potential food sources of Canada geese were collected from Kawhia harbour and the surrounding farmland in November to December 2019 (Figure 8). In each location, duplicate samples of green alga (*Ulva intestinalis*), red alga (*Gracilaria chilensis*), sea lettuce (*Ulva lactuca*), or seagrass (*Zostera muelleri*) were collected. Each duplicate contained samples for multiple plants in the area. Blades and rhizomes of *Zostera* were collected separately, although later pooled as there no significant isotopic differences between the two. Similarly, duplicate samples of pasture grass were also collected from locations around the harbour to get a wide spread of potential pasture grass sources in case of potential differences in nitrogen content due to fertilizers used on the farms. Vegetation from the Raglan region was also sampled using the same techniques. *Zostera* samples were collected from Whāingaroa Harbour in February and June 2020 and Raglan pasture grass samples were also collected in October 2020 (Figure 9) in case of variability between harbours. All vegetation samples were frozen at − 20°C until further analysis.

When ready for further processing, food source samples were defrosted and given a preliminary wash in tap water. The samples were washed another two times to remove any remaining sediment. Only non-degraded plant material from each sample was selected and left to airdry on paper towels before transferring onto aluminium foil plates. These were placed in the contherm oven at 60°C for 48 hours (Fox et al., 2009). Using scissors, samples were cut into smaller segments and ground into fine powder using a tabletop ball mill and kept in small pottles prior to isotope analysis. Three adult Yellow-bellied flounder (*Rhombosolea leporina*), originating from Kawhia harbour, were purchased from a local seafood store in August, 2020 and processed. This was necessary to assuage the concern, raised by local residents in Kawhia, that flounder form part of the diet of Canada geese and black swans. Following the protocols of Kelly et al., (2006), duplicate samples of muscle tissue (1 cm x 1 cm x 3 cm) were cut from each flounder (n = 3) with a scalpel. These samples were then dried at 60°C for 48 hours in a contherm oven before being grinded using a tabletop ball mill (Kelly et al., 2006), and kept in small pottles until isotope analysis.

Figure 8. Locations of where *Zostera muelleri*, macroalgae, and pasture grass samples were collected within the Kawhia Harbour, Westcoast of the North Island. New Zealand.

Figure 9. Locations of where *Zostera muelleri* and pasture grass samples were collected within the Whāingaroa Harbour, Westcoast of the North Island, New Zealand.

2.4. Gut content analysis

Sample containers were rinsed and drained over two stacked sieves of 500 μm and 1 mm mesh to separate stomach contents into two size classes for identification purposes. Material > 1 mm was then transferred into a large white tray under bright lights, and plant material was sorted into marine or terrestrial vegetation type, using a magnifier where necessary. As material > 1 mm tended to be dominated by a single vegetation type, the smaller fragments (1 mm to 500 μm) were assumed to be the same food source as the larger fragments. The presence of sand was notable in all samples and was removed. Samples were stored in IPA 70% prior to weighing. They were then drained and blotted dry, before weighing on an analytical balance (Sartorius CP423S) to two decimal places.

2.5. Stable isotope analysis

The $\delta^{13}C$ and $\delta^{15}N$ of samples were determined at the University of Waikato Stable Isotope Unit using a fully automated Europa Scientific 20/20 isotope ratio mass spectrometer (IRMS). Samples were first oxidised at high temperature in the furnace of an ANCA-NT GSL gas chromatograph before the gas stream was introduced to the IRMS. $\delta^{13}C$ was measured to a precision of ± 0.5% and standardised to a pre-calibrated C_4 sucrose which was cross-referenced to Pee Dee belemnite. $\delta^{15}N$ was measured to a precision of ± 1% and referenced to a urea standard that is traceable to atmospheric nitrogen.

A micro spatula and tweezers were used to transfer and weigh samples (plasma: C, 2.70 mg, N, 3.00 mg; red blood cells: C, 2.30 mg, N, 2.00 mg; feather: C 2.40 mg, N, 2.00 mg; seagrass and pasture grass: C, 2.80 mg, N, 7.00 mg; flounder: C and N, 2.20 mg) into small tin capsules (Figure 10). All weights were precise to ± 0.1 mg and were determined by running preliminary analyses to balance the sample size close to that of the reference standard, which would improve the precision of results.

Figure 10. Weighing of freeze dried blood samples into tin capsules, using a Mettler Toledo XP6 microbalance.

2.6. Statistical analysis

2.6.1. Behavioural observations

For every five-minute fixed interval, each behaviour category was converted into a proportion of the total goose activity and then averaged for each observation period. Normality and homogeneity of variances (HoV) were examined by visualising the data before running Shapiro-wilk and Levene's tests. As assumptions of HoV and normality were not met, Welch's ANOVA were used to assess the influence of group size on foraging and vigilant behaviours. Welch's ANOVA was chosen instead of Kruskal-Wallis, as it is a more robust non-parametric test for heteroscedastic data. Responses to different disturbances were also tested using Welch's ANOVA. The potential effect of disturbances encountered by geese during each observation period was assessed by observing if their responses were altered through these events. Disturbances were grouped according to their distance from the flock: 'Direct disturbances' (≤ 30 m) included agonistic behaviour from Black-backed gulls (*Larus dominicanus*), and members of the public walking on the beach with/without a dog; 'Close disturbances' (30 to 100 m) included members of the public either walking or running along the boardwalk with/without a dog; 'Distant disturbances' (> 100 m) included all watercraft in the channels (boats, kayaks, paddleboards, jet-skis), and overhead planes. Where appropriate,

the Games-Howell post-hoc test was used to identify which groups were significantly different from each other.

2.6.2. Gut contents

Data for normality and homogeneity of variances (HoV) were visualised before running Shapiro-wilk and two-sample F-tests. As data were not normally distributed, but variance was homogenous, non-parametric tests were used. In this case, Kruskal-Wallis was used to compare the mean ranks of male and female geese for both size metrics and total blotted wet weights of gut content. This test was also used to compare the blotted wet weights of the two size classes (> 1.0 mm, and < 1.0 mm > 500 μm). Spearman's rank-order correlation was used to identify the relationship strength between wingspan and length, and these size metrics with total blotted wet weight.

2.6.3. Isotopes

$\delta^{15}N$ and $\delta^{13}C$ results were analysed using R (Version 4.0.2) within the RStudio interface (Version 1.3.1093). SIDER, a fractionation estimation package for R (Healy et al., 2018) was used to predict the $\delta^{15}N$ and $\delta^{13}C$ trophic discrimination factors (TDF) for feather ($\delta^{15}N$ 5.68 ± 1.58, $\delta^{13}C$ 0.56 ± 1.35) and blood (plasma and red blood cells, $\delta^{15}N$ 4.75 ± 1.56, $\delta^{13}C$ − 0.50 ± 1.31) tissues. This method was used as no published data on controlled feeding trials (to acquire species-specific TDF), were available for the species of interest. This software uses a phylogenetic regression model based on a dataset of 409 established TDF from bird and mammal species to estimate the TDF of a consumer. Information put into SIDER was: species = "Branta_canadensis", habitat = "terrestrial", taxonomic.class = "aves", tissue = "blood" / "feather", diet.type = "herbivore". TDF (Appendix Table 2. 3) were then used in MixSIAR (Version 3.1.12, (Stock et al., 2018)) along with isotope values of food sources (pasture grass, seagrass, macroalgae) (Appendix Table 2. 4) and consumer tissues (Canada geese, black swan) (Appendix Table 2. 5, Appendix Table 2. 6, Appendix Table 2. 7). MixSIAR was used to run Bayesian mixing models and quantify the contribution of each food source to the diet of Canada geese. The model run settings of MixSIAR were at 1,200,000 (nitt; total number of iterations), 200,000 (burnin; number of iterations eliminated from the front of the chain), 500 (thin; number of consecutive iterations eliminated along the entire length of the chain) (Makowski et al., 2019). Isospace plots were created to graphically observe the isotope data before calculating the proportions of each food source contributing to the consumers' tissues. Matrix plots of the posterior dietary proportions were run separately using only Canada goose data to examine the

correlation between the different food sources. Gelman-Rubin (difference between chains < 1.05) and Geweke diagnostic (≤ 5% of each chains' standard z-score is outside of ±1.96) tests ensured the convergence of the mixing model.

Chapter Three

Results

3.1. Behavioural observations

3.1.1. Behaviour budget

Behavioural data from both observation sites (site A: Cliff street, site B: Opoturu bridge) were combined for each day to formulate the behavioural budget (Figure 11). The number of birds present at any point in time varied across observation period and days, with an average of 41 birds, and a maximum of 200 birds (seen on February 12th). Foraging was the dominant activity observed of the Canada geese on seagrass beds in Whāingaroa harbour (Figure 12A & E). The average time spent foraging across each day ranged between 66% to 94%. Vigilance was the second most observed behaviour and occurred on average 5% of their time spent on seagrass. Foraging behaviour was statistically different between observation days ($p = < 0.01$), driven predominately by the 31st of January, which was significantly different to all other days except the 30th.

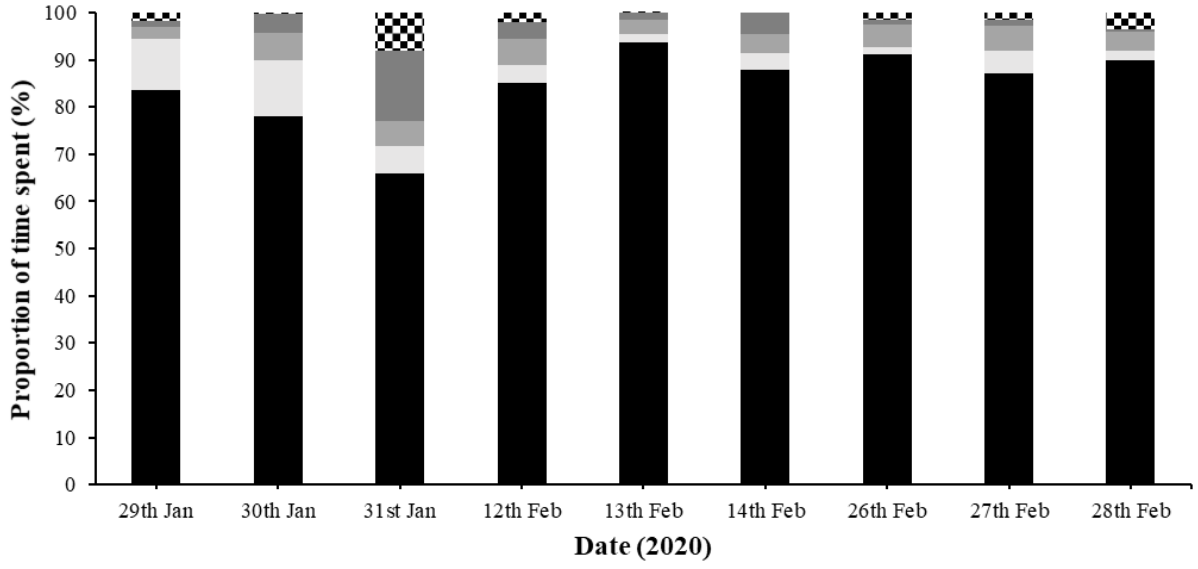

Figure 11. Average proportion of time spent by Canada geese in each behaviour category across nine days in Jan-Feb, Raglan. Foraging (■), vigilance (■), walking (■), loafing (■), and swimming (■).

3.1.2. Group size

Previous studies have found that the number of birds present in a group can alter the way they behave (Cope, 2003; Foley & Glaser, 2015; Paulus, 1988). Therefore, as foraging and vigilance were the most prominent behaviours observed, the effect of group size on these behaviours of geese at site A were examined (Figure 13). As geese from Site B were exposed to a different set of environmental conditions, observations from this site were excluded from the data. Data were organised in to three groups; small groups of 20 or fewer birds; medium groups of between 21 to 50 birds; large groups of over 50 birds.

Welch's ANOVA indicated that the proportion of foraging and vigilant behaviours was statistically significant between group sizes (Table 2). The Games-Howell test showed that this significance was between small ($n \leq 20$) and large ($n > 50$) groups of geese for both behaviours.

Table 2. Welch's ANOVA and post-hoc Games-Howell results of the proportion of geese foraging and being vigilant dependant on group size class ($\alpha = 0.05$). Significant results are given in bold.

Behaviour	df	df error	Fs	P-value	Significant Games-Howell
Foraging	2	177.97	3.85	**0.02**	Small < Large (p = **0.03**)
Vigilance	2	172.30	3.57	**0.03**	Small > Large (p = **0.02**)

Small groups of geese ($n \leq 20$) had the lowest foraging average (86%), which was significantly different from large groups of geese ($n > 50$) who foraged on average 91% of the time. Figure 13A indicates that there was still potential for 100% of geese in small groups to forage at any given point in time. Regardless of group size, the upper 75% of all observations showed a proportion of 80 to 100% of geese foraging. Smaller groups of geese also had the highest vigilance average (5%), almost double that of medium group size (3%) and significantly different from large group sizes (3%) (Figure 13B). All three box plots of vigilant geese showed a positive skew, with the lower 50% of observations from both small and medium group sizes sitting at a proportion of 0%. The median for large groups of geese was at 1%. Several outliers were displayed in both the foraging and vigilance boxplots, indicative of the behavioural variability expected in wild avian populations.

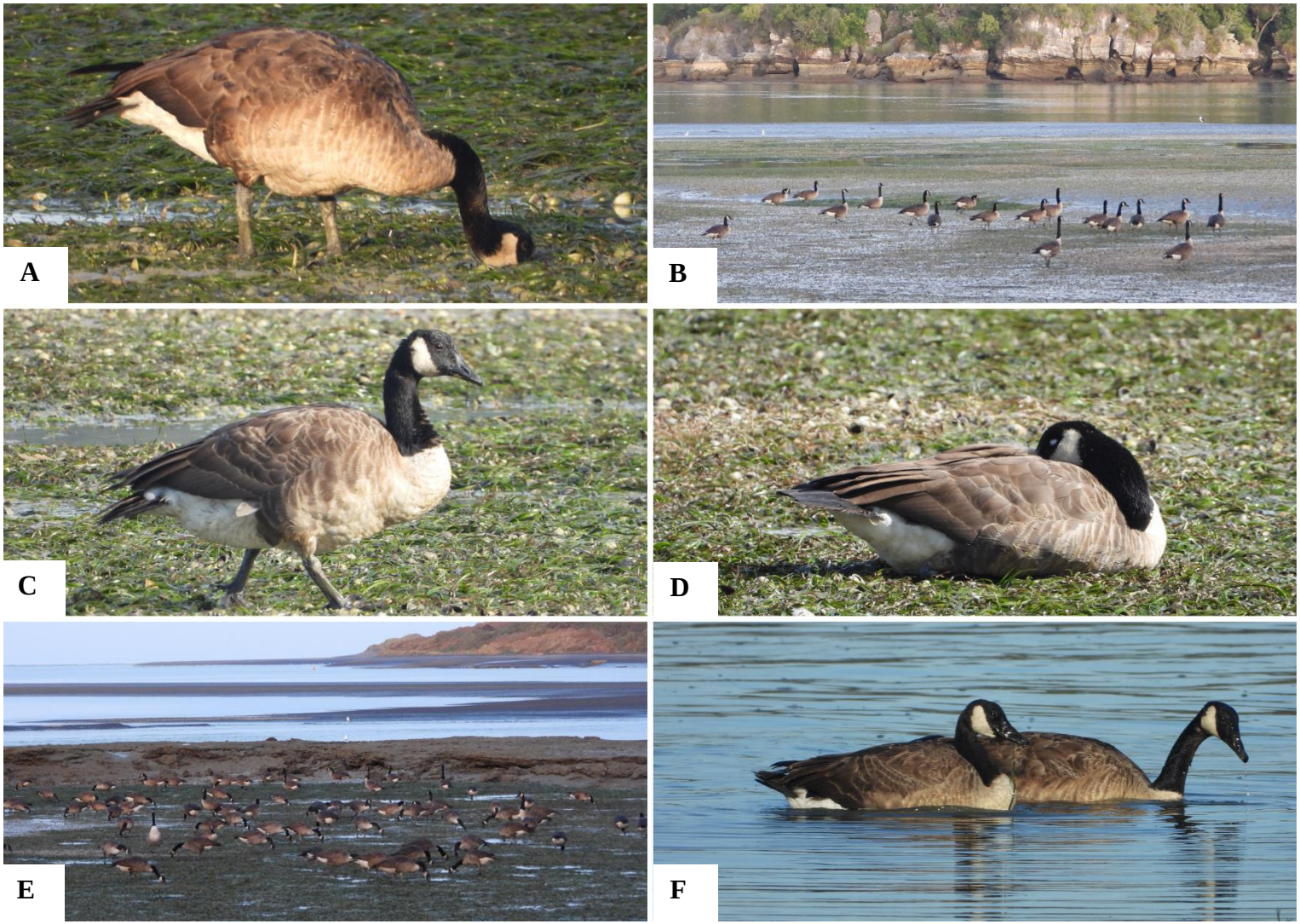

Figure 12. Examples of Canada goose behaviour in Whāingaroa Harbour showing: grubbing for *Zostera* (**A**), a small group (n < 20) displaying vigilant behaviour following a disturbance event (B), walking (C), resting (D), a large (n > 50) group foraging on *Zostera* (E), and foraging while swimming (F).

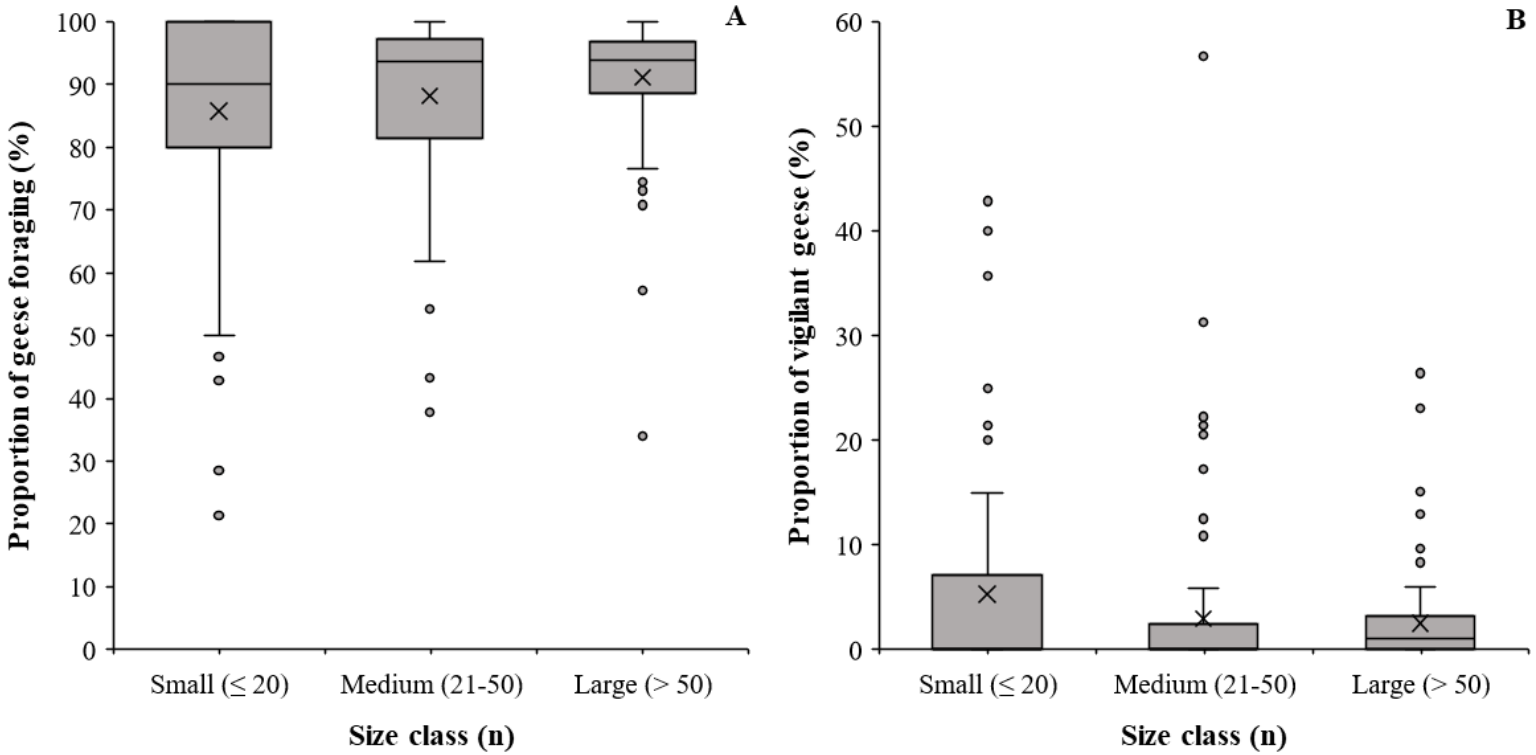

Figure 13. Boxplots showing the proportion of Canada geese foraging (A) and being vigilant (B) in different group size classes. Shaded boxes = 25th to 75th percentile; whiskers = min and max; X marks the mean; dots = outliers.

3.1.3. Disturbances

Across all three group sizes, more than 64% of the goose flock continued to forage regardless of disturbance distance (Figure 14). Direct disturbance significantly affected foraging behaviour ($p = 0.03$), with Games-Howell identifying this between small and large sized groups (Table 3). Although there was some indication that disturbances between 30 to 100 m away could alter foraging behaviours, no significant effect was found ($p = 0.08$). A high proportion of geese displaying vigilant behaviour (Figure 12B) in response to direct disturbance events was observed in small (mean: 16% ± 17%, events = 4) and medium (mean: 14% ± 24%, events = 5) sized groups. However, this result was not significant ($p = 0.94$) due to the large variances in vigilant response (Figure 15) and low frequency of disturbance events. Although geese in large group sizes did not respond to direct disturbance, no significant difference was found due to the low number of occurrences (2) over the total observations period. Irrespective of group size, Canada geese would return to foraging behaviour before the next observation period i.e., within five minutes (Appendix Figure 1. 1) unless further disturbance events occurred.

More than 30% of geese from small and medium sized groups took flight in response to direct disturbance (Figure 16). However, the variance in this flight response for small (± 36%) and medium (± 25%) group sizes was high. All other disturbances caused less than 5% flight response. Welch's ANOVA indicated there was statistical significance ($p = 0.01$) between different group sizes in response to close disturbance (Table 3). Games-Howell post-hoc specified this significant difference between medium and large groups of geese ($p = 0.02$).

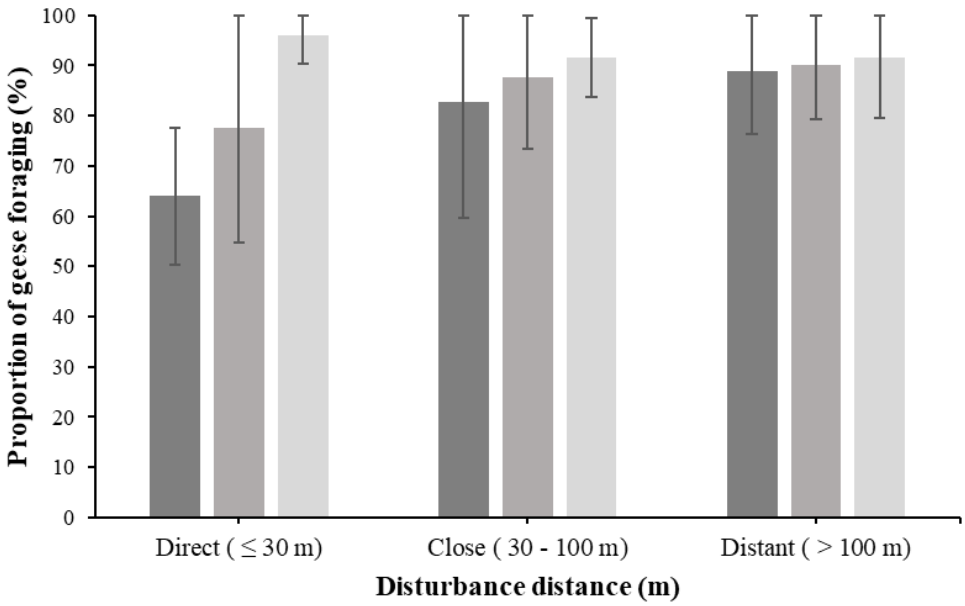

Figure 14. The mean proportion of Canada geese in each group size (small ■: n ≤ 20, medium ■: 21 > n < 50, large ■: n > 50) that forage during a disturbance event (direct: ≤ 30 m, close: 30 to 100 m, distant: > 100 m). Error bars indicate one standard deviation.

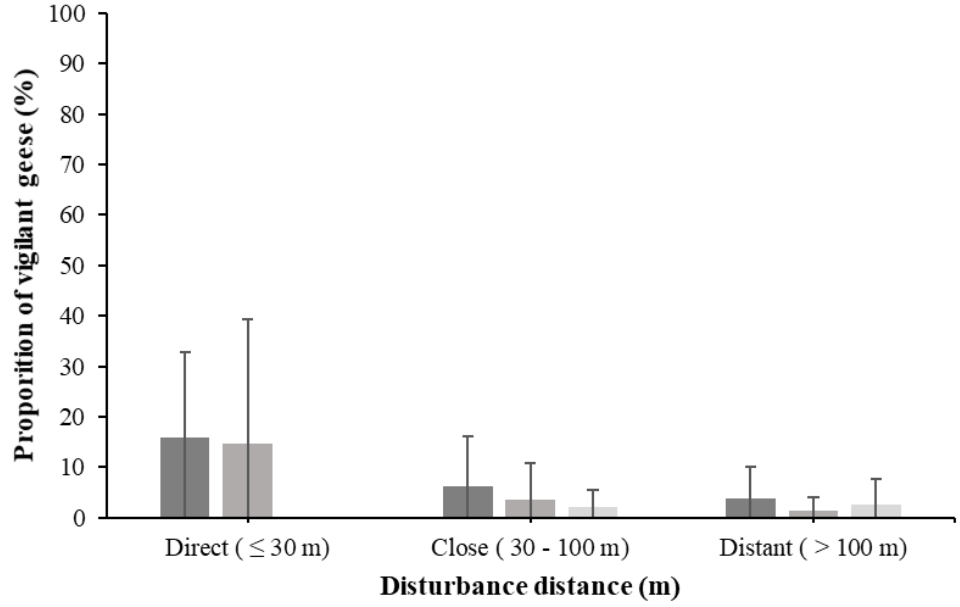

Figure 15. The mean proportion of Canada geese in each group size (small ■: n ≤ 20, medium ■: 21 > n < 50, large ■: n > 50) that were vigilant during a disturbance event (direct: ≤ 30 m, close: 30 to 100 m, distant: > 100 m). Error bars indicate one standard deviation.

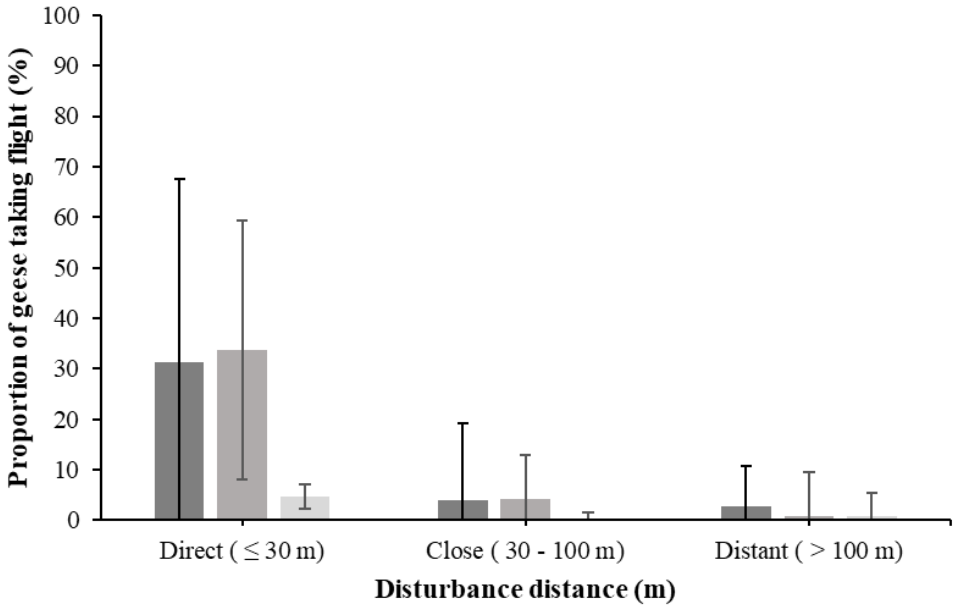

Figure 16. The mean proportion of Canada geese in each group size (small ■: n ≤ 20, medium ■: 21 > n < 50, large ■: n > 50) that took flight in response to a disturbance event (direct: ≤ 30 m, close: 30 to 100 m, distant: > 100 m). Error bars indicate one standard deviation.

Table 3. Welch's ANOVA and post-hoc Games-Howell results of the response (foraging, vigilance, flight) of Canada geese in each group size (small: $n \leq 20$, medium: $21 > n < 50$, large: $n > 50$) to different disturbance distances (direct: ≤ 30 m, close: 30 to 100 m, distant: > 100 m). ($\alpha = 0.05$). Significant values are represented in bold font.

Behaviour	Disturbance distance	df	Df error	Fs	P-value	Significant Games-Howell
Foraging	Direct	2	5.23	7.68	**0.03**	**Small < Large (p = 0.03**)
	Close	2	55.87	2.59	0.08	
	Distant	2	47.10	0.32	0.73	
Vigilance	Direct	1	6.92	0.01	0.94	
	Close	2	55.08	2.67	0.08	
	Distant	2	42.89	1.80	0.18	
Flight	Direct	2	4.71	3.65	0.11	
	Close	2	45.75	4.97	**0.01**	**Med > Large (p = 0.02**)
	Distant	2	42.42	0.50	0.61	

3.2. Gut content analysis

The gut contents, comprised of the proventriculus and gizzard, indicate the most recent (within 2 hours) ingested food by Canada geese. In nearly all cases, gut contents were made up of one food source only, with the exception of two geese from Raglan; one which had a combination of sea lettuce (*Ulva lactuca*) with *Zostera*, and the other sea lettuce with pasture grass. As seen in Table 4, pasture grass was the dominant food source consumed by geese from Kawhia (n = 22) and Raglan (n = 22).

Table 4. The food sources found in the gut contents of Canada geese from Kawhia and Raglan harbours.

	Kawhia (n)	**Raglan (n)**
Pasture grass	22	22
Zostera	2	1
Three-square bulrush	1	0
U. lactuca + *Zostera*	0	1
U. lactuca + pasture grass	0	1
Empty	8	1

Although gut contents were separated into two size classes for ease of identification, the total blotted wet weight (BWW) of gut contents was analysed for each harbour to get a more accurate representation of total food consumption by geese. Prior to analysing the differences in BWW, one outlier (female Raglan goose) was removed from the dataset due to an unusually high total BWW of 27.07 g. The average gut content weight from Raglan geese (mean: 5.53 g ± 5.64) was greater than gut contents from Kawhia geese (mean: 3.59 g ± 4.42), but was not significantly different ($p = 0.16$) due to the large variances around each mean. Both box plots (Figure 17) show positive skew, with the lower 75% of total BWW data less than 10 g.

Table 5. Mean gut content weights of the biomass size classes > 1.00 mm, and < 1.0 mm to > 500 μm with total blotted wet weight, for Canada geese from Kawhia and Raglan harbours. Standard deviation is given in parentheses.

	Kawhia (g) (±SD)	**Raglan (g) (±SD)**
> 1.00 mm	2.63 (± 3.36)	4.32 (± 4.76)
< 1.0 mm to > 500 μm	0.97 (± 1.20)	1.21 (± 0.98)
Total blotted wet weight	3.59 ± (4.42)	5.53 (± 5.64)

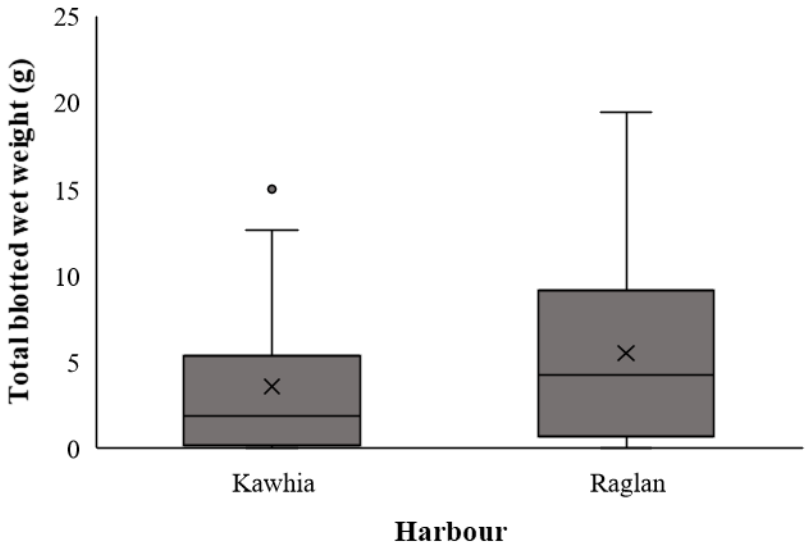

Figure 17. The blotted wet weight of gut content within each biomass size class for Canada geese from Kawhia and Raglan harbours. Shaded boxes = 25th to75th percentile; whiskers = min and max; X marks the mean.

3.2.1. Potential gender differences in recent consumption

A linear relationship between wingspan and body length was observed across the entire sample population of Canada geese (Figure 18). Pearson's correlation also indicated a significantly strong coefficient (r = 0.76) between wingspan and body length, indicating either metric was an appropriate proxy for bird size. A significant difference in size between male and female geese was identified through Kruskal-Wallis (p = < 0.01). The average male had a wingspan of 161 cm and body length of 103 cm, whereas the average female had a smaller wingspan of 156 cm and body length of 97 cm. All geese with a wingspan greater than 167 cm and/or a body length greater than 106 cm were males, indicating that males tend to grow larger in size than females (Figure 18).

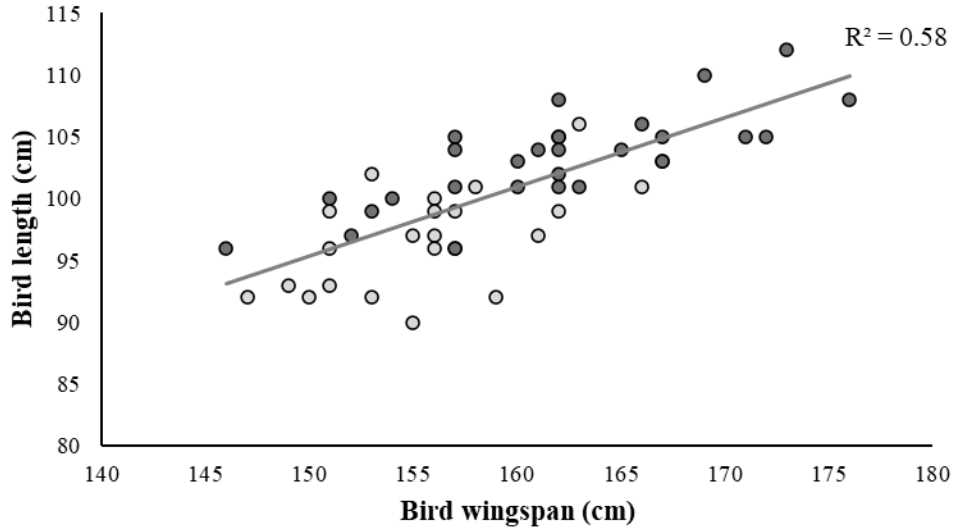

Figure 18. Coefficient of variation between bird wingspan and bird length for male (●) and female (○) Canada geese.

As larger geese may have a greater gut capacity, the difference in total blotted wet weight (BWW) between males and females was evaluated (Figure 19). However, the average gut content of males (5%) and females (4%) were not significantly different from each other (p = 0.67). Both box plots were positively skewed, indicating that data constitute a higher frequency of larger BWW. Using bird length as the proxy for overall size, Pearson's correlation test indicated no clear relationship between total BWW and bird size across the total population (r = 0.17, p = 0.25).

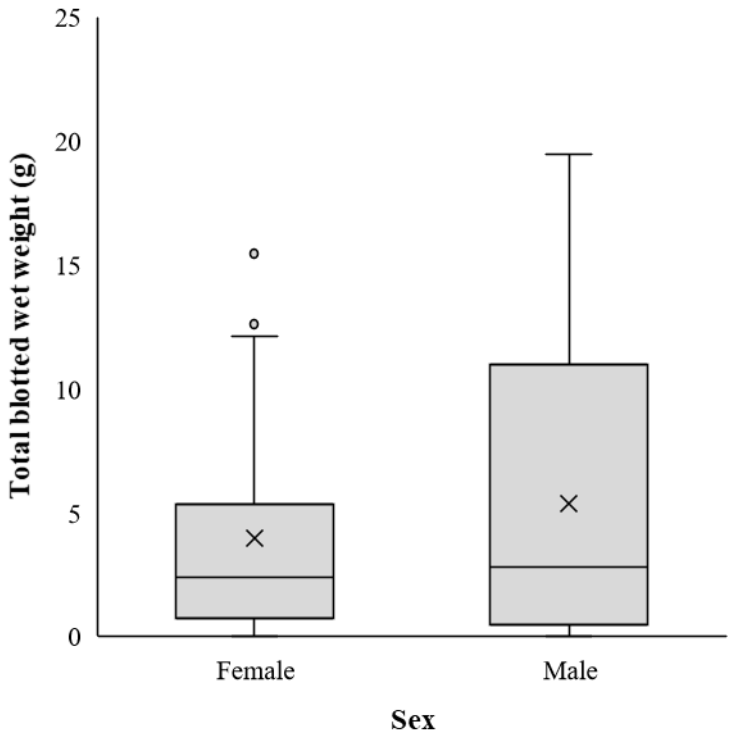

Figure 19. Total blotted wet weight of gut content for male and female Canada geese. Shaded boxes = 25th to75th percentile; whiskers = min and max; X marks the mean; dots = outliers.

3.3. Stable isotope analysis

Pasture grass, macroalgae and seagrass (*Zostera*) were isotopically distinct potential food sources for Canada geese. *Zostera*'s signature combines both seagrass blades (δ^{15}N, 6.39‰ ± 0.53, δ^{13}C, − 11.21‰ ± 1.05, (mean ± 1 standard deviation)) and roots and rhizomes (δ^{15}N, 5.71‰ ± 0.62, δ^{13}C, − 11.36‰ ± 0.78), as no significant difference was identified between their δ^{13}C ($p = 0.75$). Samples from both harbours were pooled for analysis as no differences in δ^{15}N and δ^{13}C of pasture and *Zostera* were found between locations ($p > 0.05$) (Table 6). Similarly, potential algal food sources (*Gracilaria chilensis*, *Ulva lactuca,* and *Ulva intestinalis*) had similar carbon and nitrogen signatures (mean δ^{15}N, 7.36‰ ± 0.40, mean δ^{13}C, − 18.78‰ ± 0.64) and so were pooled and analysed as 'macroalgae'.

Table 6. Mean of *Zostera* and pasture grass samples from Kawhia and Raglan harbours. There was no significant difference of δ^{15}N and δ^{13}C between the two harbours ($p = > 0.05$). Standard deviation is given in parentheses.

Harbour	Food source	δ^{15}N Mean (SD)	δ^{13}C Mean (SD)
Kawhia	*Zostera*	5.89‰ (± 0.66)	−11.39‰ (± 0.99)
Raglan	*Zostera*	6.52‰ (± 0.43)	−10.98‰ (± 0.51)
Kawhia	Pasture grass	3.18‰ (± 3.24)	−27.39‰ (± 3.60)
Raglan	Pasture grass	2.66‰ (± 2.93)	−29.18‰ (± 1.29)

To address concerns from local iwi and the community, that Canada geese were feeding on juvenile flounder, a culturally and economically valuable estuarine species, samples from yellow-bellied flounder (*Rhombosolea leporina*) were examined (δ^{15}N 13.01‰ ± 0.77, δ^{13}C − 16.86‰ ± 1.57) as a potential food source. However, a preliminary assessment of the proportional contribution of each food source with MixSIAR (Stock & Semmens, 2016) clearly revealed that *R. leporina* were not being utilised as a food source by any of the Canada geese or black swans sampled (Appendix Figure 2. 1). Therefore, these data were not included in further mixing model runs.

Three different tissues were used in separate mixing models to identify which food sources contributed to the diet of Canada geese across three different time frames. Plasma was assumed to reflect the diet three to four days prior to sampling, and red blood cells were assumed to reflect the diet three to four weeks prior to sampling. Primary flight feathers were assumed to reflect the diet at the time they were grown (late December and early January, summer season).

Isotopic values from black swan tissues were also included in each mixing model to enable dietary comparison between the two species, as black swans are known seagrass consumers (Santos et al., 2012; Santos et al., 2013).

3.3.1. Plasma

Plasma has the fastest turnover rate of the tissues sampled, and represents the diet three to four days prior to sampling. Trophic discrimination factors (TDF) used in the mixing model were estimated by the R package SIDER (Healy et al. 2018). The TDF, + 4.75‰ for $\delta^{15}N$ and − 0.50‰ for $\delta^{13}C$, derived from SIDER for 'blood', were used for both plasma and red blood cell (RBC) samples (see section 2.6.3). The assimilated diet of Canada geese from both Kawhia and Raglan harbours assessed from plasma tissue was predominately pasture grass (Figure 20). Raglan geese showed less spread in the data ($\delta^{15}N$ 4.93 to 6.80‰ and $\delta^{13}C$ − 29.85 to − 28.30‰) than the isotope values from Kawhia geese, but although Kawhia isotope values had a greater range, many were closer to the mean for the pasture grass signature ($\delta^{15}N$ 4.27 to 8.39‰ and $\delta^{13}C$ − 25.02 to − 29.77‰).

Plasma probability density plots (Figure 23), showed that the dominant food source for geese from Kawhia (Figure 23A) was pasture grass, making up a mean of 87% (± 4% SD) of their diet, with a 95% credible interval (CI) of 79 to 94%. Similarly, Raglan geese (Figure 23B) also showed pasture grass as the dominant food source (mean: 93% ± 3%, CI 86 to 98%). While there was a small contribution (11%) of *Zostera* to the plasma of Kawhia geese, for Raglan this was less than 5% (Table 7). The probability density distributions for plasma were narrow, indicating minimal variation between individuals. However, there was some overlap in the distribution of *Zostera* and macroalgae, which was more prominent in Raglan than in Kawhia geese.

The correlation between the different food sources was examined (after exclusion of black swan data) to assess the reliability of determining which food sources were being consumed together. A strong negative correlation ($r = -0.73$) between these two food sources for Canada geese, indicated that their dietary proportions, although minimal, were difficult to distinguish from each other (Appendix Figure 2. 2A). Diagnostic tests confirm convergence of this model run at a "very long" length (Stock & Semmens, 2016). Convergence was confirmed with the Gelman-Rubin diagnostics, for which out of 91 variables, none exceeded 1.01 (Appendix Table

2. 8). The Geweke diagnostic (a standard z-score), had less than 5% of the 91 variables in the three chains >1.96, also confirming convergence (Appendix Table 2. 9).

In contrast to the geese, isotope values of black swans primarily fell within the range of marine vegetation (*Zostera* and macroalgae), with the exception of two individuals whose results were more closely aligned with a pasture grass diet (Figure 20). The primary food source assimilated into the plasma tissue of black swans was *Zostera* (mean: 75% ± 6%), as clearly shown in the density plot (CI: 62 to 84%) (Figure 23C). This probability density distribution showed no overlap with the other food sources.

3.3.2. Red blood cells

Red blood cells (RBC) represent the diet three to four weeks prior to sampling. The same TDF from SIDER (estimated for 'blood' + 4.75‰ for $\delta^{15}N$, − 0.50‰ for $\delta^{13}C$), was also used for RBC. Isotopic values from Kawhia and Raglan geese were distributed close to the isotopic signature of pasture grass (Figure 21). Although the spread of $\delta^{15}N$ and $\delta^{13}C$ values are similar between RBC and plasma tissues, the plasma values were situated closer to the mean of the pasture grass signature ($\delta^{15}N$, 3.00‰ ± 3.05, $\delta^{13}C$ − 27.99‰ ± 3.09) (Figure 20) in comparison with RBC values that were more nitrogen depleted (Figure 21).

Raglan geese values were clustered between a range of 4.93 to 6.80‰ ($\delta^{15}N$) and − 29.85 to − 28.30‰ ($\delta^{13}C$). This indicated there was little variability in food selection during the winter/spring sampling period (August to September) even though the geese were collected from several different locations around the harbour. Pasture grass was also the dominant food source assimilated into RBC for Kawhia (mean: 88%, CI 80 to 95%) (Figure 24A) and Raglan (mean: 93%, CI 86 to 98%) with *Zostera* and macroalgae contributing less than 10% to geese from both harbours (Table 8).

The probability density distributions (Figure 24) for Canada geese indicated that although there was minimal variance between the RBC of individual birds, the two food sources were not clearly distinguishable due to the overlap between *Zostera* and macroalgae. A strong negative correlation was present ($r = -0.69$), meaning that the exact proportion of the two food sources could not be made reliably (Appendix Figure 2. 2B). If *Zostera* was being consumed at the top of its probability range, then macroalgae would likely be at the bottom of its probability range or vice versa (Phillips et al., 2014). This model, run at a "very long" length (Stock & Semmens,

2016), remained appropriate for this data as convergence was achieved. For the Gelman-Rubin diagnostics, out of 91 variables, none exceeded 1.01 (Appendix Table 2. 8). The Geweke diagnostic (a standard z-score), had none of the 91 variables in the three chains >1.96 (Appendix Table 2. 9).

Zostera and macroalgae contributed a high proportion to the diet of black swans in Kawhia Harbour, with the exception of three individual swans whose tissue isotopes were closer to pasture grass (Figure 21). *Zostera* was the predominant food source contributing an average 71% (CI: 55 to 81%) to black swans' diet. The probability density distributions for each food source assimilated into black swan RBC (Figure 24C) showed minimal overlap, but were comparatively wider than the density curves of Canada geese, due to the variability present in individual diet selection. The average contribution of each food source to Canada geese and black swan RBC (Table 8), were all almost identical to plasma samples (Table 7). This indicates that the diets of these waterfowl were consistent across the two time periods (days to weeks).

3.3.3. Primary feathers

An examination of the food sources assimilated during the Canada geese and black swans moult period (mid-December to late January), was provided through primary feather tissues, using the feather-specific fractionation values estimated by SIDER (δ^{15}N +5.68‰, δ^{13}C + 1.58‰). Isotopic values of Canada geese from both Kawhia and Raglan harbours ranged from 4.23 to 11.13‰ for δ^{15}N, and − 19.58‰ to − 27.02‰ for δ^{13}C. Although widely dispersed, in comparison to plasma and RBC plots (Figure 20, Figure 21), there still appeared to be a strong affinity towards pasture grass as the predominant food source (Figure 22). Only one goose (from Raglan), was dispersed closer towards the isotopic range for macroalgae (δ^{15}N 11.13‰, δ^{13}C − 19.70‰). The dietary proportion of each food source in the probability density plots (Figure 25) showed that pasture grass contributed an average proportion of 77% to the diet of Kawhia geese (CI: 66 to 89%), and 75% to Raglan geese (CI: 61 to 90%). Kawhia geese had a higher contribution of *Zostera* (16%) than Raglan geese (6%), and Raglan geese had a higher proportion of macroalgae in their diet (19%) (Table 9).

Although it was clear that the geese were consuming mainly pasture grass, a moderately strong negative correlation ($r = -0.43$) in the posterior probability between *Zostera* and macroalgae and a strong negative correlation ($r = -0.64$) between *Zostera* and pasture grass indicated that

these food sources may have been difficult to distinguish from each other in these feather tissue samples (Appendix Figure 2. 3). Although the balance between the three food sources remains unclear (Parnell et al., 2013), convergence was achieved and the model remains an appropriate fit for the data. For the Gelman-Rubin diagnostics (Appendix Table 2. 8), out of 88 variables, none exceeded 1.01. The Geweke diagnostic (a standard z-score), had less than 5% of the 88 variables in the three chains >1.96 (Appendix Table 2. 9).

A shift from *Zostera* and macroalgae towards pasture grass in the black swan isotopic values was observed in Figure 22. Fewer black swan feather samples were available, in comparison to plasma and RBC (Figure 20, Figure 21), due to primary feather samples only being obtainable from adult male swans. Most black swan values showed an overlapping isotopic distribution with the geese, except for two swans whose results were positioned within the *Zostera* range. For the faster metabolising tissues, plasma and RBC, these two swan samples were previously located within the pasture grass signature (Figure 20, Figure 21). Similar to Canada geese, pasture grass was the dominant food source assimilated in the swan feathers (Table 9) (mean: 38%, CI 24 to 54%).

Canada geese and black swan feather probability density distributions (Figure 25) were all wider than plasma and RBC curves (Figure 23, Figure 24). This indicated that there was greater variability between the diet of individual birds, during the time of feather growth. These curves also presented overlapping distributions, suggesting variability in the assimilation of these food sources between different birds.

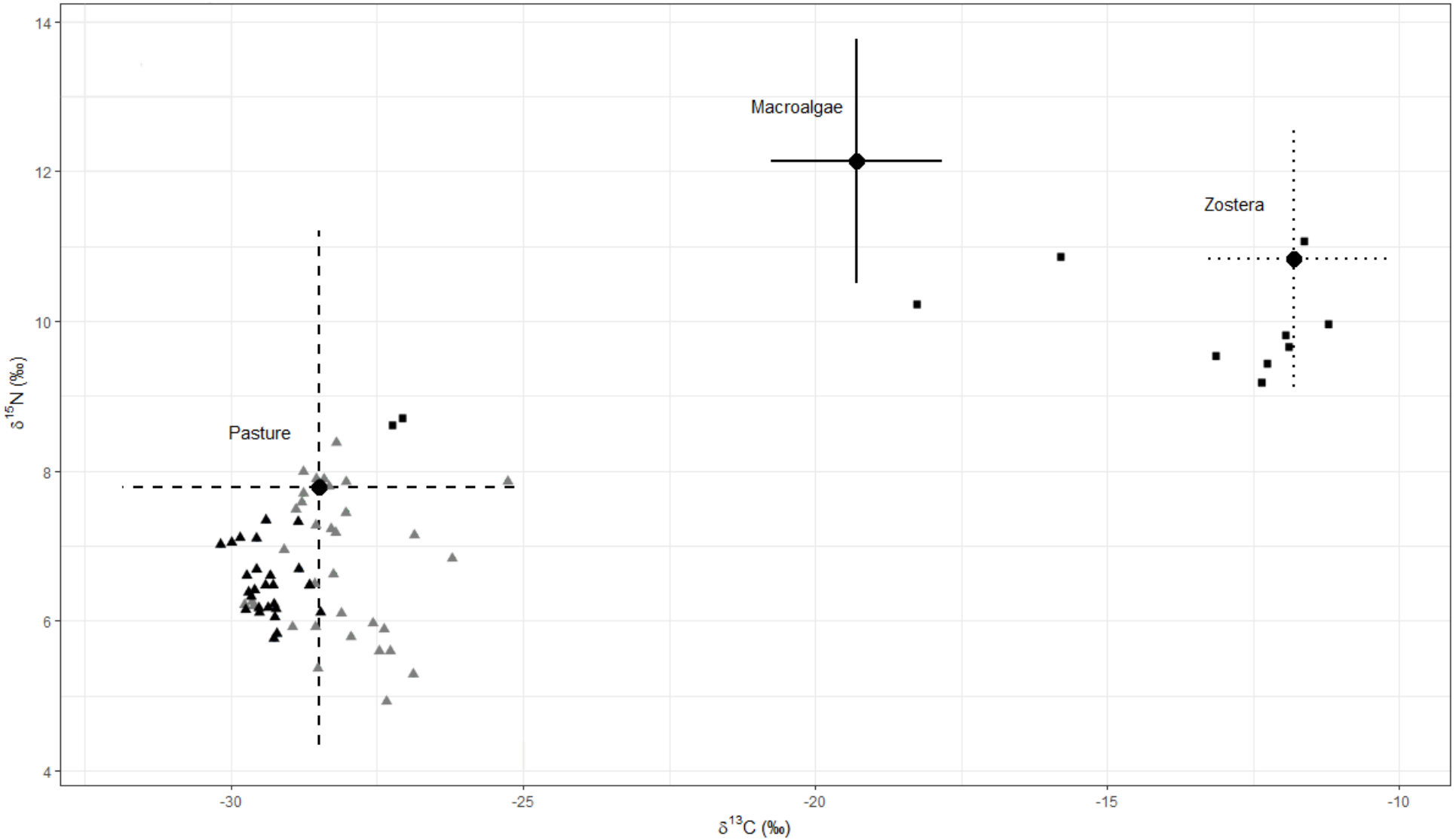

Figure 20. Isospace plot of the isotopic signatures from Canada geese and black swan plasma tissue. Error bars show the standard deviation of the mean (●) of each food source which have been adjusted with TDF. Consumer information is displayed for black swans (■), Kawhia Canada geese (▲), and Raglan Canada geese (▲).

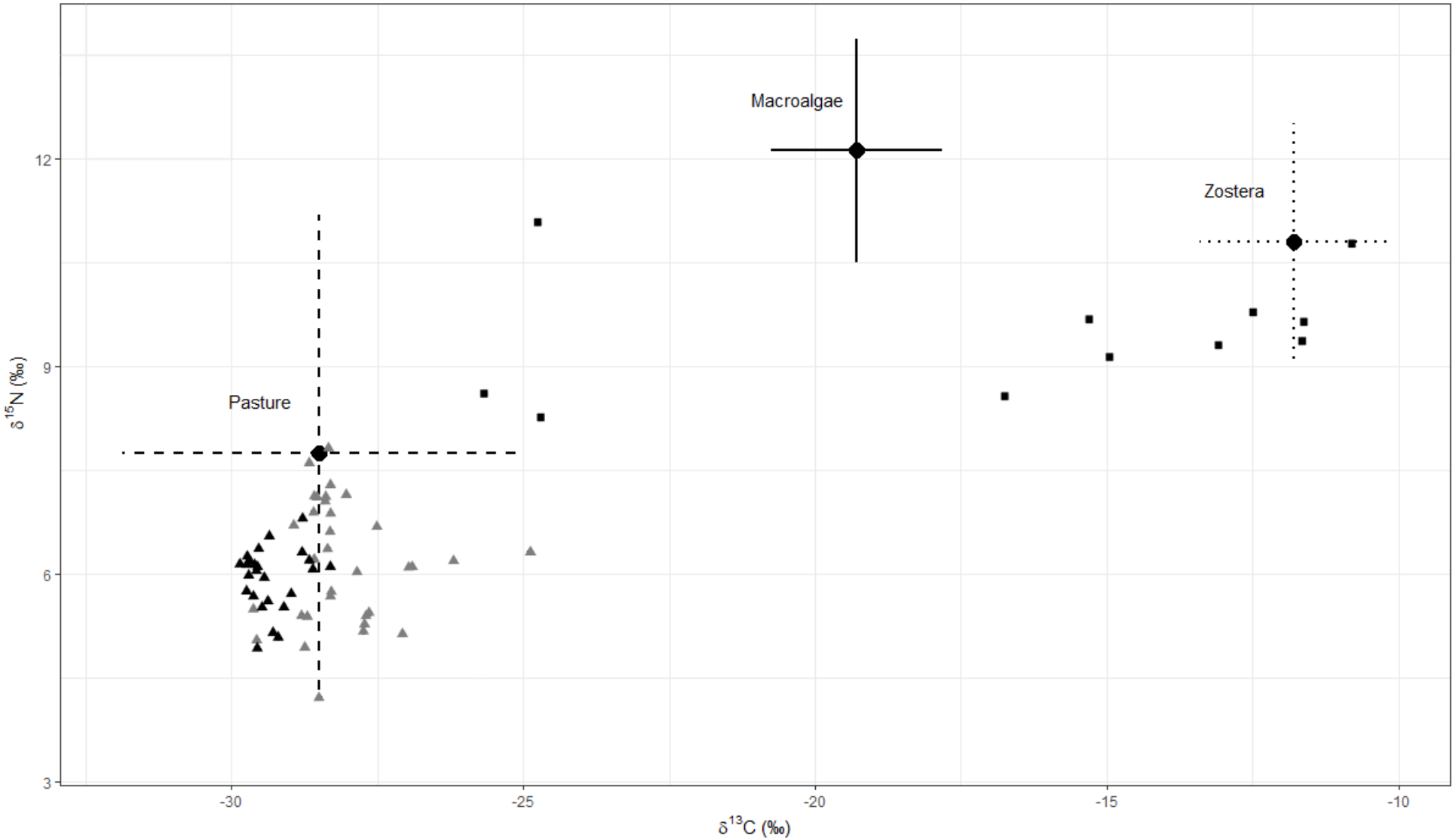

Figure 21. Isospace plot of the isotopic signatures from Canada geese and black swan red blood cells. Error bars show the standard deviation of the mean (●) of each food source which have been adjusted with TDF. Consumer information is displayed for black swans (■), Kawhia Canada geese (▲), and Raglan Canada geese (▲).

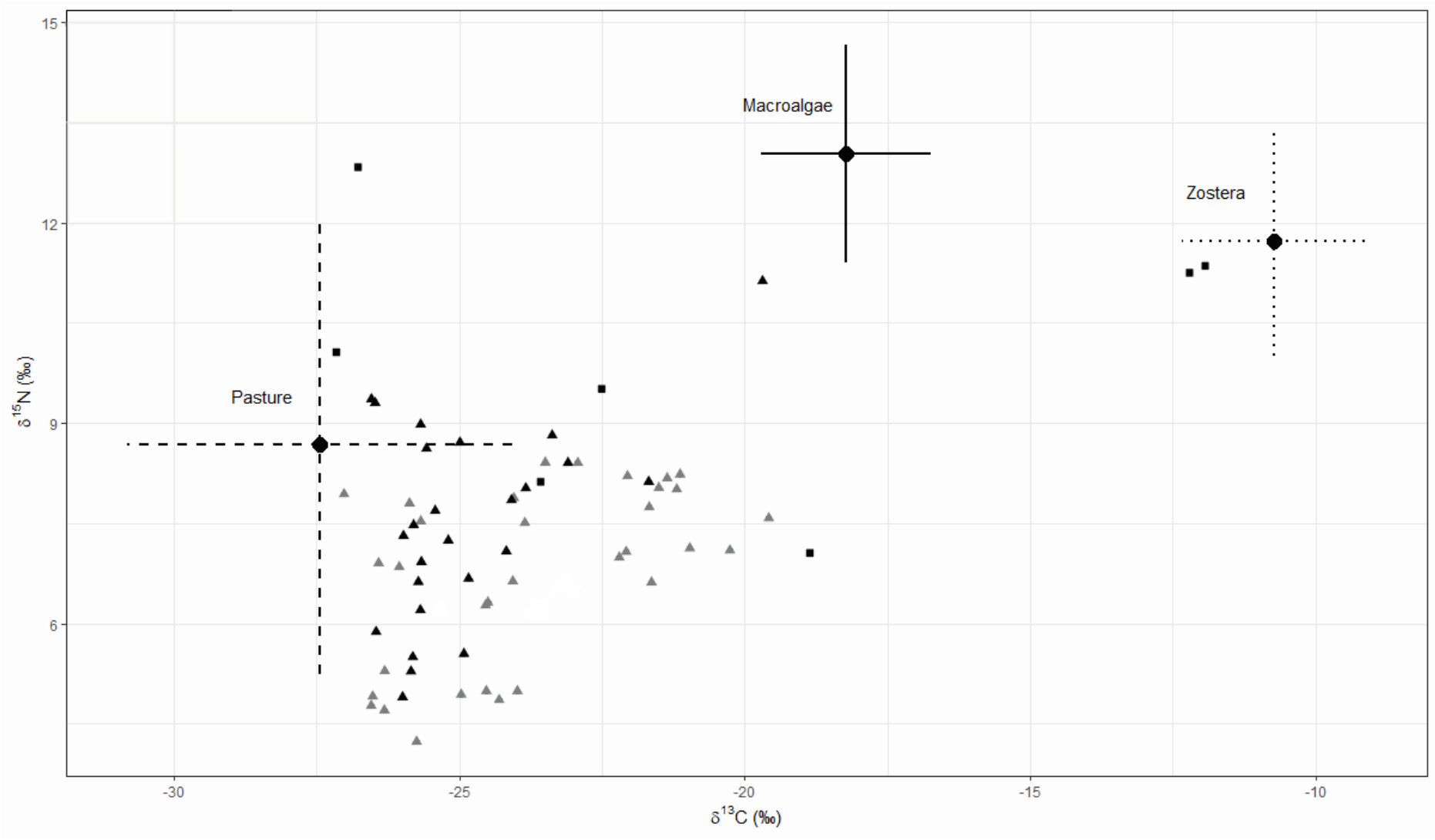

Figure 22. Isospace plot of the isotopic signatures from Canada geese and black swan feathers. Error bars show the standard deviation of the mean (●) of each food source which have been adjusted with TDF. Consumer information is displayed for black swans (■), Kawhia Canada geese (▲), and Raglan Canada geese (▲).

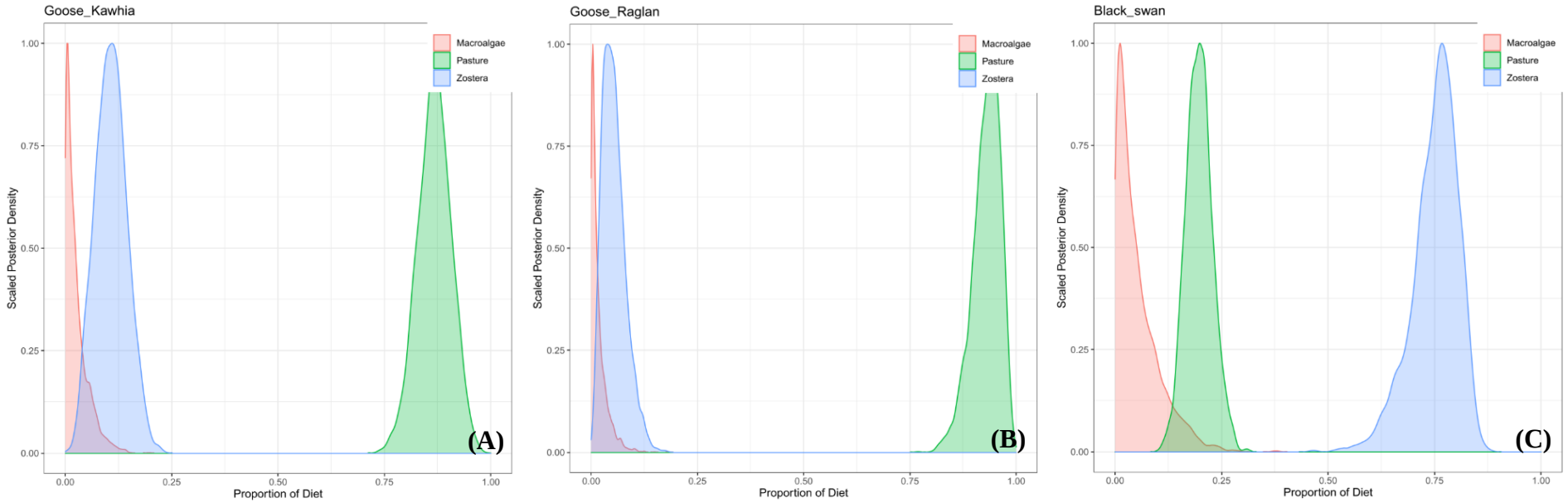

Figure 23. Density plots of dietary proportion for plasma samples from Kawhia Canada geese (A), Raglan Canada geese (B), Kawhia black swans (C).

Table 7. Mean proportion of each food source to the plasma of Canada geese in Kawhia and Raglan harbours, and black swans in Kawhia Harbour. Standard deviation is given in parentheses.

	Macroalgae	**Pasture grass**	***Zostera***
	Mean (± SD)	**Mean (± SD)**	**Mean (± SD)**
Black swan: Kawhia	0.05 (± 0.05)	0.20 (± 0.03)	0.75 (± 0.06)
Canada goose: Kawhia	0.03 (± 0.03)	0.87 (± 0.04)	0.11 (± 0.04)
Canada goose: Raglan	0.02 (± 0.02)	0.93 (± 0.03)	0.05 (± 0.03)

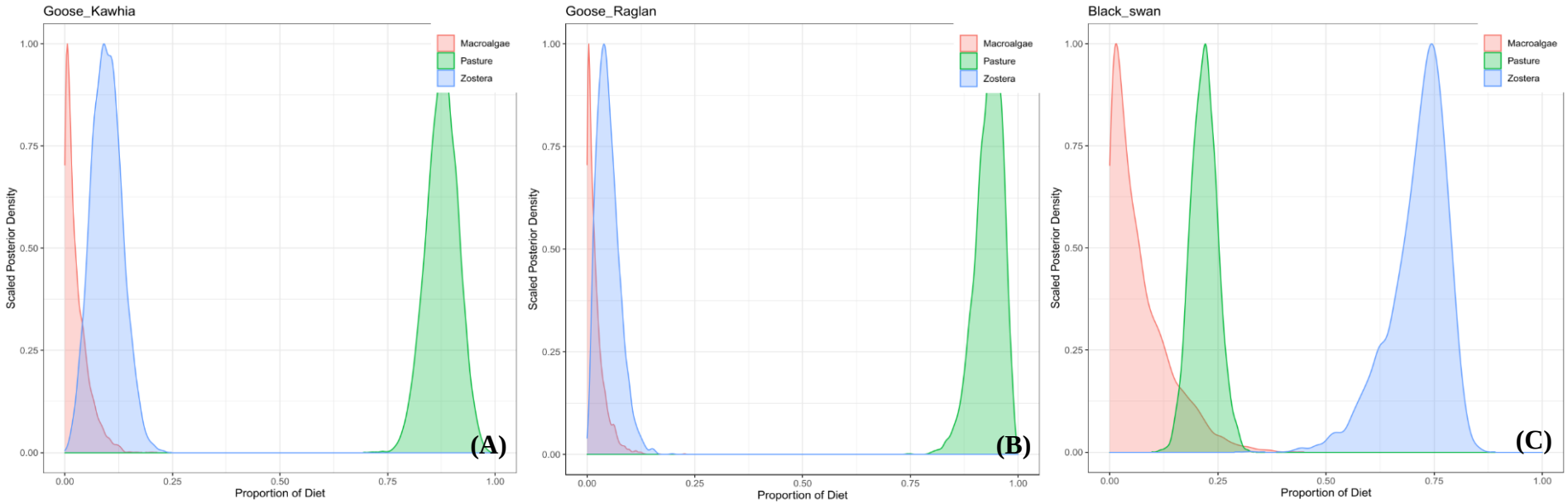

Figure 24. Density plots of dietary proportion for red blood cell samples from Kawhia Canada geese (A), Raglan Canada geese (B), Kawhia black swans (C).

Table 8. Mean proportion of each food source to the red blood cells of Canada geese in Kawhia and Raglan harbours, and black swans in Kawhia Harbour. Standard deviation is given in parentheses.

	Macroalgae	**Pasture grass**	***Zostera***
	Mean (± SD)	**Mean (± SD)**	**Mean (± SD)**
Black swan: Kawhia	0.07 (± 0.07)	0.22 (± 0.03)	0.71 (± 0.07)
Canada goose: Kawhia	0.03 (± 0.03)	0.88 (± 0.04)	0.10 (± 0.04)
Canada goose: Raglan	0.02 (± 0.02)	0.93 (± 0.03)	0.05 (± 0.03)

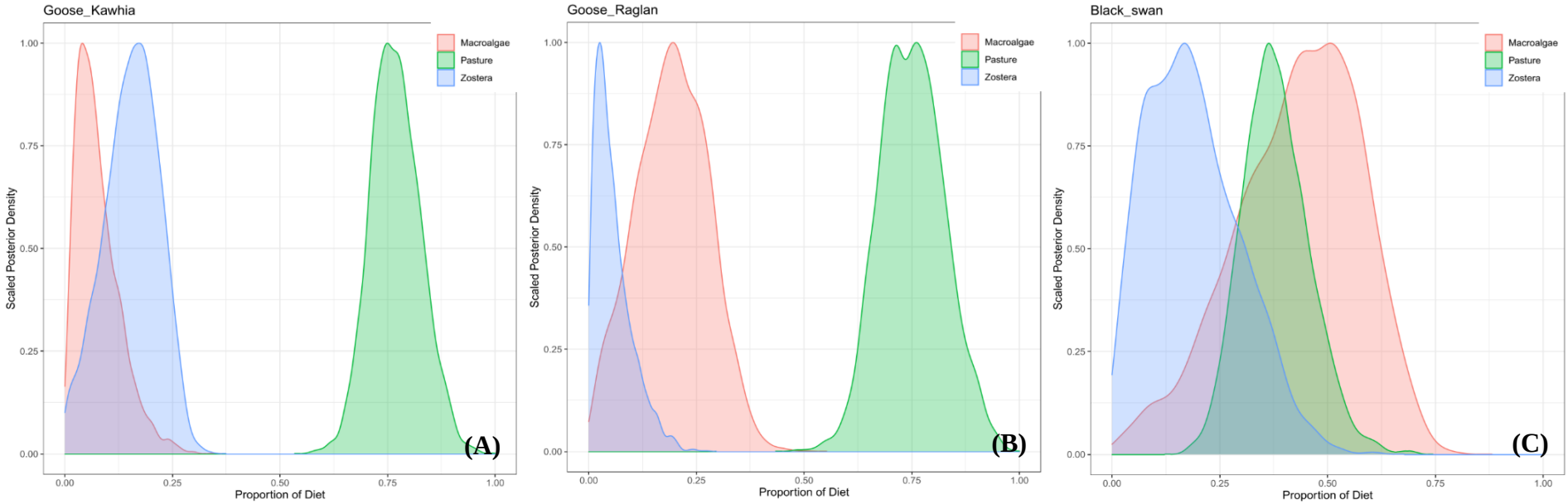

Figure 25. Density plots of dietary proportion for primary feather samples from Kawhia Canada geese (A), Raglan Canada geese (B), Kawhia black swans (C).

Table 9. Mean proportion of each food source to the feathers of Canada geese in Kawhia and Raglan harbours, and black swans in Kawhia Harbour. Standard deviation is given in parentheses.

	Macroalgae	**Pasture grass**	***Zostera***
	Mean (± SD)	**Mean (± SD)**	**Mean (± SD)**
Black swan: Kawhia	0.43 (± 0.14)	0.38 (± 0.08)	0.19 (± 0.11)
Canada goose: Kawhia	0.08 (± 0.05)	0.77 (± 0.06).	0.16 (± 0.06)
Canada goose: Raglan	0.19 (± 0.08)	0.75 (± 0.07)	0.06 (± 0.04)

Chapter Four
Discussion

Seagrass (*Zostera muelleri*) beds are ecologically important habitats that deliver key ecosystem functions and services, often supporting biodiverse marine communities (Barnes & Hughes, 1999; Hemminga & Mateo, 1996). These habitats are in a state of general decline across New Zealand (Matheson & Schwarz, 2007; Turner, 2007) and are vulnerable to further impacts due to increasing anthropogenic, biotic and climate related disturbances (Himes-Cornell et al., 2018; Matheson & Schwarz, 2007; Santos et al., 2013; Turner, 2007; Turner & Schwarz, 2006a; Turner & Schwarz, 2006b; Zabarte-Maeztu et al., 2020). Since 1986, the numbers of Canada geese have increased from 460 to more than 10,000 geese in the Waikato region alone. The most recent estimated number of Canada geese present the two estuaries under investigation is approximately 1,300 birds in Raglan and 1,000 birds in Kawhia (Smith, 2019). I investigated the use of *Zostera* as a food resource by Canada geese (*Branta canadensis*) in order to better understand the potential impacts of grazing on seagrass beds in west-coast, North Island, estuaries. This study used behavioural observations, gut content analysis, and stable isotope analysis to:

- Determine how much time Canada geese (*Branta canadensis*) spend foraging while on seagrass (*Zostera muelleri)* beds, and whether behaviour changed in response to the proximity of different disturbances or different group sizes.

- Identify how much of the most recently ingested food by *B. canadensis* is comprised of *Zostera*, and whether this is dependent on bird size or sex.

- Evaluate the proportion of *Zostera* assimilated into *B. canadensis* tissues, relative to other food sources, and whether a temporal shift in diet selection (days to months) could be detected through isotopic analysis of three different tissue types (plasma, red blood cells, and primary feathers).

4.1. Behavioural observations

Formulating a behavioural budget is a valuable method to provide necessary data in foraging ecology studies (Supanwanid et al., 2001). In my study, behavioural observations found that Canada geese spent an average of over 85% of their behavioural budget foraging whilst on the *Zostera* beds in Whāingaroa Harbour. However, their usage of *Zostera* may be seasonal, as Canada geese were only present during January and February, and not in June. The observation of foraging as the dominant behaviour in my study is consistent with other behavioural studies (Foley & Glaser, 2015; Paulus, 1988; Win, 2001). Foraging may incorporate a large proportion of their behavioural budget due to high energy and protein demands, following the breeding and moult period (Foley & Glaser, 2015). Although my behavioural observations were limited to the daytime only, Canada geese can be active during both the day and night time (Jorde & Owen, 1988).

4.1.1. The influence of group size on Canada geese behaviours

Canada geese are strongly social and influenced by the movement of their conspecifics, generally foraging and moving around in family flocks (Foley & Glaser, 2015). The flocks I observed on seagrass in Whāingaroa Harbour ranged from 8 to 200 individuals and flock size significantly influenced the proportion of geese foraging on the seagrass at any one time ($p = 0.02$); the more geese present, the greater the proportion of geese foraging. This was negatively associated with vigilant behaviour, which decreased in larger group sizes. Larger groups of geese were able to increase their time spent foraging by relying on the vigilance of the entire group (Foley & Glaser, 2015; Inger et al., 2006b). Even though this result seems intuitive due to increased detection of potential threats, numerous studies have been unsuccessful in showing this relationship (Beauchamp, 2008; Foley & Glaser, 2015). One possible reason for this difference may be that my study has been conducted on wild geese, while previous avian studies were mostly performed in controlled settings, potentially altering natural behaviours (Beauchamp, 2008), that are often variable among and within species (Paulus, 1988). It is also possible that foraging and vigilant behaviours are not mutually exclusive, and that individual geese are constantly scanning their environment for disturbances while they forage (Foley & Glaser, 2015).

4.1.2. The effect of disturbances to Canada geese behaviours

Although not the main focus of my study, the response of Canada geese in each group size (small: $n \leq 20$, medium: $21 > n \leq 50$, large: $n > 50$) to disturbance distances (direct: ≤ 30 m, close: 30 to 100 m, distant: > 100 m) was assessed. Direct disturbance events (≤ 30 m) were found to have a significant effect on geese foraging behaviour, specifically between small and large group sizes ($p = 0.03$). Fewer geese would forage when directly disturbed due to the potential immediate threat to their safety. Previous studies have shown that in response to repetitive disturbances, Canada geese either gradually ignore or display learned avoidance in response to repetitive disturbances (White, 1986). As such, hunting strategies may often lose their effectiveness over time. In Whāingaroa Harbour, geese appeared to have habituated to disturbances of passing vehicles, planes, watercraft, and most occurrences of people walking on the footpath. However, this may not be the case for Canada geese on *Zostera* beds in Kawhia as these are more remote.

Generally Canada geese are sensitive to unpredictable and erratic disturbances (Chudzinska et al., 2013), which are often produced by other species. In my study, humans walking on the beach (Site A) appeared to illicit a greater vigilant response, especially when walking with a dog. Dogs presented the geese with unpredictable behaviour outputs, and were often unleashed so had the potential to run through the flock. Black-backed gulls (*Larus dominicanus*) are the largest gull species in New Zealand (Seabrook-Davison et al., 2008) and were observed swooping to attack the geese. It is not surprising that these gulls would pose a threat to Canada geese, as there have been numerous reports of *L. dominicanus* attacking other animals including, tuatara, lambs and seabird chicks (Seabrook-Davison et al., 2008). Of course, due to recreational hunting, humans pose the biggest threat to the survival of these geese, as adults have no natural predators in New Zealand (White, 1986).

Although vigilance was influenced by group size, my study did not find that disturbance distance significantly influenced this behaviour. This is likely due to a low frequency of vigilant responses during disturbance events and the infrequency of the events themselves during my observations. The lack of vigilant behaviour may also suggest that the geese had either habituated to a vast majority of the disturbance events (Chudzinska et al., 2013) due to the proximity of the seagrass beds to Raglan township, or responded with another behaviour. Flight response was one such behaviour and caused more than 30% of the geese in small and medium sized groups to leave their group during direct disturbance events. This dropped below 10%

during close disturbance events in large groups, with a significant difference between medium and large groups of geese ($p = 0.02$). Again, this is likely due to the increased safety in numbers in larger flocks.

Further data on the influence of different disturbances to the behaviours of Canada geese is needed to confirm the trends observed in this study. A controlled behavioural study, such as that conducted by Lord et al., (1996), who examined the effect of different disturbance approaches on the northern New Zealand dotterel (*Charadrius obscurus aquilonius*), are required in order to determine which disturbance type would be most effective at disrupting the foraging behaviours Canada geese on *Zostera* beds.

4.1.3. The implications of Canada geese foraging on seagrass beds

The methods Canada geese use to forage *Zostera* may have serious implications on the survival and resilience of *Zostera* beds in West Coast estuaries. Consistent with previous studies (Cade, 2016; Ganter, 2000), behavioural observations of geese in Whāingaroa Harbour revealed that they use several foraging methods to remove *Zostera* from the sediment (Appendix Table 1. 1). Geese removed Zostera from the sediment by dabbling, grubbing, and trampling behaviour. This can increase turbidity, rework the upper sediment layer, and reduce sediment stability (Nacken & Reise, 2000), likely influencing the distribution of biota within the habitat (Marklund & Sandsten, 2002; Nicastro & Bishop, 2013). The presence of seagrass provides faunal communities with physical protection, habitat complexity, and increased food availability (Leopardas et al., 2014). Consequently, the loss of seagrass blades and roots/rhizomes may negatively affect biodiversity and community composition within this habitat (Leopardas et al., 2014; Mills & Berkenbusch, 2009). Grubbing behaviour can cause seagrass fragmentation and has been found to lower macrofaunal diversity, in comparison with continuous undisturbed *Zostera* beds (Dixon, 2009; Mills & Berkenbusch, 2009). However, this result may vary dependent on the functional traits of macrofauna species inhabiting the seagrass beds (Leopardas et al., 2014) and the physical conditions associated with the seagrass habitat (Dixon, 2009). A study on black swan in Golden Bay, New Zealand, found that foraging on *Zostera* predominantly affected seagrass biomass, with a less pronounced effect on the macrofaunal community structure (Dixon, 2009). In one location, although macrofaunal diversity decreased, overall abundance increased, potentially due to reduced interspecific competition (Dixon, 2009).

The root system of seagrass beds may be weakened or entirely removed through foraging disturbances, resulting in exposed feeding pits (Macreadie et al., 2014; Marklund & Sandsten, 2002; Santos et al., 2013). Patches of bare sediment within seagrass beds may alter bed-flow dynamics, turbulent kinetic energy and increase the potential for erosion (van der Heide et al., 2012). This can have negative feedbacks on the remaining seagrass through increased turbidity in the water column which may limit primary production (Drylie et al., 2018). A reduction in seagrass biomass also reduces its photosynthetic potential and can hinder its overall growth (Maschinski & Whitham, 1989; Santos et al., 2013). However, studies disagree on whether these feeding pits are detrimental or not, with some suggesting that they facilitate seedling propagation, and further seagrass growth by reducing mud accretion (Ganter, 2000; Nacken & Reise, 2000; Supanwanid et al., 2001; Zipperle et al., 2010).

Previous studies in the Northern Hemisphere have found that annual seagrass loss due to herbivory by waterfowl, ranges between 20 to 98% of the total standing biomass (Balsby et al., 2017; Clausen et al., 2012; Nacken & Reise, 2000). In the Northern Hemisphere, the presence of waterfowl is associated with migration, so these birds remain on the seagrass beds anywhere from two weeks to several months (Clausen et al., 2012; Nacken & Reise, 2000; Santos et al., 2012). A study on *Zostera* grazing by Brent geese (*Branta bernicla*) and Wigeon (*Anas penelope*) in the Northern Wadden sea, Germany, suggested that seasonal herbivory was essential for the persistence of *Zostera* beds. Geese foraging behaviours such as grubbing and trampling reduced mud accretion, and intraspecific competition, enabling recovery of seagrass within the following growing season (Nacken & Reise, 2000). However, a similar pattern was not observed in Tauranga Harbour (New Zealand), on *Zostera* beds grazed by black swan (*Cygnus atratus*), with biomass removal between 20 to 80% (Santos et al., 2012). This is likely due to individual environmental conditions present between locations that contribute to seagrass growth and reproduction.

Although not a common occurrence in New Zealand's *Zostera* beds, the presence of flowering shoots for seed germination (sexual regeneration), occurs at low densities between October to June, peaking in late summer (Ramage & Schiel, 1998; Santos & Matheson, 2017). Flowering is associated with plant coverage > 75%, and stimulated by high biomass, especially of roots/rhizomes (de Kock et al., 2016; Ramage & Schiel, 1998; Santos & Matheson, 2017). However, this is also often the peak presence of waterfowl on seagrass beds (Santos et al., 2012; Smith, 2019; Spurr & Coleman, 2005). The foraging of Canada geese on flowering

Zostera beds, which have been observed in Whāingaroa Harbour (de Kock et al., 2016), may limit sexual reproduction of this plant, affecting its genetic diversity and fitness (Santos & Matheson, 2017).

Recovery and expansion of *Zostera* beds in New Zealand predominantly occurs by rhizome encroachment (asexual regeneration). Elongation of rhizomes largely occurs at seagrass edges (Turner, 2007; Turner & Schwarz, 2006b), but the growth and productivity of *Zostera* is often higher in the centre of the beds (Turner, 2007). This is likely due to the edges of numerous feeding pits created by waterfowl foraging in the centre of the beds where biomass is greater. Although recovery by rhizome encroachment can take anywhere from 18 to 35 weeks (Macreadie et al., 2014), full recovery could take 2 to 4 years; assuming a constant recovery rate and disregarding all other stressors (Santos et al., 2013). Vegetative growth of rhizomes is therefore important in frequently disturbed habitats for local expansion of seagrass (Ramage & Schiel, 1998). However, behavioural observations of Canada geese in Whāingaroa Harbour revealed that the entire *Zostera* plant was often removed, not just the blades. Geese may have been targeting the rhizomes because of their higher sugar and starch content than the rest of the *Zostera* plant (Santos et al., 2012). Accessibility to these nutrients in a digestible form, may provide geese with the energy required for fat deposition (Cadieux et al., 2005), particularly for breeders following the moult period, and for goslings that have high growth rates.

Given my observations indicated flocks may spend up to 6 hours on *Zostera* beds, foraging on average 85% of the time, there is an assumption that birds are also defaecating in these environments. An individual Canada goose may defaecate anywhere between 28 to 200 times day^{-1} (Buij et al., 2017; Manny et al., 1994), with dry weight approximating 2 to 4% of their total body mass (60 to 200 g day^{-1}) (Buij et al., 2017). Bird guano has a long history of being used as fertiliser due to its high nitrogen (N) and phosphorus (P) content; essential nutrients for plant growth (Fujita & Kameda, 2016). Angiosperms take up nutrients from the sediment rather than directly form the water column and positive impacts on *Zostera* growth at a local scale have been observed in oligotrophic systems (Powell et al., 1991) as a result of bird faeces. In New Zealand estuaries increased nutrient loading from guano may instead exacerbate the pervasive problem of eutrophication (Smith, 2019).

Furthermore, New Zealand seagrass beds with low ambient biomass and low macrofaunal diversity have been shown to be less resilient to enrichment (Gladstone-Gallagher et al 2018)

which may indicate those with greater grazing pressure may be the most vulnerable. Having said this, the contribution of both N and P from guano is likely to be relatively minor in comparison to other sources e.g., land runoff (Buij et al., 2017; Mitchell & Wass, 1995; Raffaelli, 1999), except in circumstances of very high population densities (Mitchell & Wass, 1995). Further research is required to elucidate some of these complex relationships.

4.2. Gut contents of Canada geese

Behavioural observations in Whāingaroa Harbour in January to February 2020, showed that Canada geese were foraging on *Zostera* during low-tide emersion in the mornings. Although the geese were not present on the *Zostera* beds later in the year, insight into their immediate diet (within 2 hours) was achieved by identifying vegetation fragments in their gut contents (August to September). Canada geese are effective at grinding vegetation, but the nature of their typically fibrous diet still enables evaluation of remaining plant fragments (Buchsbaum et al., 1986; Supanwanid et al., 2001). Canada geese from Kawhia and Raglan possessed gut contents comprised predominantly of pasture grass (70% and 88%, respectively). Almost all stomachs contained one food source only, with the exception of two geese from Raglan, one which had a combination of sea lettuce with seagrass, and the other had sea lettuce with pasture grass. This demonstrated that although geese generally fed on pasture grass during the sampling period (Coleman, 2008), they are capable of exploiting other food sources when they are available (Buchsbaum & Valiela, 1987; Chudzinska et al., 2015; Eaton et al., 2017; Sedinger & Raveling, 1988), and select for specific parts of plants (Foley & Glaser, 2015; Washburn & Seamans, 2012).

One Canada goose gizzard contained the brown seeds of three-square bulrush (*Schoenoplectus pungens*). This sedge species often grows alongside *Zostera* patches in Kawhia (Graeme, 2012a), which may help explain this dietary anomaly. It may be that the goose opportunistically feed on *S. pungens*, while already in the area to forage on *Zostera*. This highlights that gut analysis data has its limitations, as some plant species will be more easily digested (i.e., *Zostera*) due to higher water and lower fibre content. Consequently, these species may be identified at a lower frequency within the gut contents than other vegetation (Inger et al., 2006a; Inger et al., 2006b). Vegetation fragments identified in the stomach may also be biased as all birds had to be shot on farmland, their limited gut retention and random timing of their death. The average blotted wet weight of gut contents in this study measured 4.02 g (Kawhia) and

5.53 g (Raglan), but based on calculations from Owen. (1972), and Manny et al. (1994), the total amount of vegetation that an individual Canada goose could consume varies between 450 to 2,200 g day^{-1}. This scales to a maximum of 2.2 and 2.9 tonnes of vegetation per day, based on the most recent (2018) population numbers for the Kawhia and Raglan regions, respectively.

4.3. Stable isotope analysis

4.3.1. Diet of Canada geese during New Zealand's winter and spring months

Behavioural observations revealed an absence of Canada geese on the *Zostera* beds in Whāingaroa Harbour at the start of the winter period (June), and evaluation of their gut contents indicated pasture grass as the dominant food source being consumed at that point in time. Further insight into the diet of Canada geese during winter and spring (July to November) was achieved using stable isotope analysis. This provided quantitative data on the food sources being assimilated into multiple tissues to enable the diet of geese to be observed on a temporal scale (Hobson et al., 1993; Hobson & Clark, 1992a; Inger et al., 2006a). Using stable isotope analysis presents its own set of challenges due to a lack of existing avian data, with virtually none on herbivorous waterfowl (Hahn et al., 2012). This approach requires in-depth knowledge of the species in question, including an understanding of the fractionation process that occurs as food sources are assimilated into various tissues. Although acquiring species-specific fractionation data can be difficult and time consuming, involving long-term controlled feeding experiments (Grecian et al., 2015; Hahn et al., 2012; Parnell et al., 2013; Unkovich et al., 2001), it would be beneficial to reduce any unexplained variation in dietary proportions of Canada geese in New Zealand.

Of the three tissues sampled in this study (plasma, red blood cells (RBC), and primary feathers), plasma has the fastest metabolic turnover rate and represents the diet of Canada geese three to four days prior to sampling. RBC tissues have a slower turnover rate so represent their diet three to four weeks prior to sampling. Within my study, plasma and RBC tissues yielded very similar isotopic results (Table 7, Table 8), suggesting that separation of blood samples was not necessary, as the diets of Canada geese were consistent across the two time frames.

All blood samples were taken from Canada geese between July and November, providing dietary information of geese in winter and spring, during which they are known to spend their time inland near freshwater lakes and wetlands, feeding predominantly on surrounding pasture

grass (Choney et al., 2014; Spurr & Coleman, 2005). Similar to the gut contents, the patterns observed in plasma and RBC tissues displayed a diet of predominantly pasture grass, during the winter and spring seasons (mean: 87 to 93%, SD ± 4%).

4.3.2. Temporal shifts in diet and the use of endogenous reserves

Unlike the plasma and RBC plots, which showed clear distribution of Canada geese isotope values within the pasture grass signature, the distribution of feather isotope values (Figure 22) were dispersed away from pasture grass towards other undetermined dietary source or sources with higher $\delta^{13}C$ values. Canada geese primary feathers are moulted and regrown late December to early January on the spring breeding grounds, which are generally inland (Munafo & Gibbs, 2012; Spurr & Coleman, 2005). Although my study showed that the dominant food source contributing to the primary feathers of Canada geese was pasture grass, and was consistent between harbours (Kawhia 77% ± 6%, Raglan 75% ± 7%), stored C and N resources in the geese's bodies may have been utilised.

The use of endogenous energy resources may enable the rapid regrowth of primary feathers (within 30 to 35 days) following the energetically stressful breeding and moult period (Bearhop et al., 2002; Fox et al., 2009; Hahn et al., 2012; Hobson et al., 1993; Hobson & Clark, 1992b; Lewis et al., 2010; Munafo & Gibbs, 2012; White, 1986; Zanden & Rasmussen, 2001). In this scenario, isotopic values would be expected to be distributed between pasture grass and the food sources that had contributed to their reserve tissues (Fox, Hobson, & Kahlert, 2009); similar to the isotope values plotted in Figure 22. The reliability of the stable isotope mixing model depends on the accuracy of incorporating all food sources that are being consumed by the particular species studied (Hobson et al., 1993; Vanderklift & Ponsard, 2003) and that the presumed TDF are correct. However, birds may potentially use endogenous reserves in primary feather production (Fox et al., 2009). In this situation, it is beneficial (although not often practical), to have knowledge on all potential habitats visited by the study species, as endogenous reserves may be built in muscle or fat stores throughout the year (Fox et al., 2009; Murphy, 1996). Although the contribution of endogenous reserves into feather formation is not well understood (Bearhop et al., 2002; Fox et al., 2009; Hobson & Clark, 1992a), some argue that it is of minor significance, due to birds having limited to no physical reserves following the energetically costly breeding season (Murphy, 1996). The variability in feather isotope values between individual Canada geese may in fact be a genuine representation of their diets,

as breeding pairs of geese may be spreading out across the landscape to nest and moult (White, 1986), with some potentially breeding near marine food sources.

4.3.3. Potential diet shifts during New Zealand's drier summer and autumn months

Behavioural data indicated Canada geese utilise *Zostera* beds in the summer period but were absent in winter. Primary feathers are already fully regrown by the time they arrive on these habitats in summer, which may explain why feather data did not show a strong association with marine food sources. Unfortunately, Covid-19 lockdown restrictions meant sampling in this critical post-moult period was not possible and therefore I cannot conclusively demonstrate a reliance on *Zostera* in their diet at this time of year. Following their breeding season, geese are believed to make a habitat shift from terrestrial pasture grass to a *Zostera* due to nutritional quality and quantity of food sources available, and dietary requirements of the bird (Buchsbaum & Valiela, 1987; Choney et al., 2014; Inger et al., 2006b; Nolet et al., 2002; Parnell et al., 2013; Supanwanid et al., 2001; Vickery et al., 1995; White, 1986).

Geese move towards coastal lakes and estuaries during the summer-autumn period, as drought conditions limit inland water sources and pasture grasses become unpalatable (Choney et al., 2014; Spurr & Coleman, 2005; Win, 2001). It is likely that geese select food sources to meet a certain minimum threshold in water content during the dry summer and autumn seasons (Buchsbaum & Valiela, 1987). An official drought was recorded during the 2020 summer season throughout the Waikato (Waikato Regional Council, 2020), so pasture grass was likely to be particularly dry and dead. It is likely that, *Zostera* would be more palatable than pasture grass at this time, with greater water and energy content, lower fibre, and more soluble carbohydrates (Buchsbaum & Valiela, 1987; Clausen et al., 2012; Dokter et al., 2018). During the winter period, *Zostera* patch size often declines due to cooler wetter weather and more frequent storm events that enhance wave action and sedimentation (Turner & Schwarz, 2006b). Dietary shifts between these two habitats have been observed by Brent and Canada geese in the Northern Hemisphere, and by black swans in New Zealand and Australia (Choney et al., 2014; Santos et al., 2012; Santos et al., 2013).

4.3.4. Variation in dietary requirements and food selection of Canada geese

Canada geese may adjust their foraging patterns in order to meet high growth demands in spring and summer, and high energy demands during autumn and winter (Buchsbaum & Valiela, 1987; Cadieux et al., 2005; Clausen et al., 2012; Gadallah & Jefferies, 1995; Jorde & Owen,

1988; Paulus, 1988; Supanwanid et al., 2001; White, 1986; Win, 2001). Geese are behaviourally flexible (Jorde & Owen, 1988; Win, 2001), and very mobile (Win, 2001), so have the potential to forage in several different locations. Variability in the dietary requirements of individual geese may be present due to differences in sex and age (Paulus, 1988). Although no significant difference was observed in the gut contents of geese within this study, a previous study has found that female geese tend to feed more than males (Paulus, 1988). This pattern was only present in breeding females, which had greater requirements to replenish their reserves before and after the breeding period (Cadieux et al., 2005; Cope, 2003; Gadallah & Jefferies, 1995; Raveling, 1979).

Although sexually mature at two years of age most Canada geese do not start breeding until three to four years old (White, 1986). Non-breeders are likely to have greater access to food resources as they are not required to incubate (females) or guard the nest (males) (White, 1986). This could lead to differences in dietary requirements and selection between younger non-breeders and older breeding geese; the latter which are limited to the food resources available surrounding the nest. Variation in dietary selection of breeding geese may also exist due to nests being built isolated from other breeding pairs (White, 1986), potentially in habitats with different food sources available.

4.3.5. Robustness of the stable isotope mixing models

In order to accurately model the diet of individual organisms, it is important to have a good understanding of species behaviour, habitat range and potential food sources utilised (Parnell et al., 2013). The identification of vegetation fragments within gut contents aid in determining the important food sources required for stable isotope analysis. As the food sources adjusted with TDF, and their standard deviations encompass all individual consumers (Figure 20, Figure 21, Figure 22), we can be confident that the correct food sources were used in the mixing models. Furthermore, the black swan isotope values show a clear switch in diet between pasture grass and *Zostera* in the feather and blood (plasma and RBC) tissues, indicating that we would be capable of seeing this pattern in the Canada geese if it were present. The potential consumption of flounder (*Rhombosolea leporina)* was eliminated due to gut content analysis and isotope results clearly indicating this resource was not being utilised (Appendix Figure 2. 1). This is a positive outcome in regards to the conservation of juvenile flounder in New Zealand estuaries, as many are endemic, economically valuable, and culturally significant (Constable, 2014). Although, Canada geese are characterized as herbivorous waterfowl

(Buchsbaum & Valiela, 1987; Eaton et al., 2017; Sedinger & Raveling, 1988), there have been previous reports of geese opportunistically foraging on fish in Iowa, USA (Eaton et al., 2017). In my study, it is more likely that these organisms were in fact misidentified trematode flatworms (flukes) which are internal parasites often associated with the liver (David Klee, pers. comm. Auckland/Waikato Fish and Game, Hamilton).

4.3.6. Appropriate trophic discrimination factors in stable isotope analysis

The application of assumed TDF is often the weakest link in isotope studies, and even small inaccuracies can lead to misleading results (Bond & Diamond, 2011; Caut et al., 2009; Cherel et al., 2005; Jardine et al., 2003). Many studies have used discrimination factors based on the nearest taxonomic group, even when there are clear habitat and dietary differences (Bond & Diamond, 2011; Caut et al., 2009). There is a large deficit of established species-specific discrimination factors in published literature, largely due to the difficulties in conducting controlled feeding experiments. A review of avian TDF (plasma, RBC, feather) in previous studies found limited values for herbivorous waterfowl (Appendix Table 2. 10). However, SIDER (Healy et al., 2018), was established to estimate TDF for avian and mammalian species, when these feeding experiments are not applicable. This package in R incorporates all established avian and mammalian discrimination factors from previous dietary studies in cooperation with their phylogenetic trees. SIDER was used in this study as no previous studies have established discrimination factors for Canada geese. As the SIDER program is relatively new to stable isotope mixing models, it would be beneficial to establish TDF from controlled feeding trials to see how they compare. This would require experimental trials of caged geese to be fed a diet of *Zostera* or pasture grass, for a minimum of one year, in order to establish appropriate TDF.

I can be relatively confident that the TDF used in the plasma and RBC model were adequate because both isospace plots had consumer values surrounding the mean value of the pasture grass signature. However, this is not apparent in the feathers, which may be due to several scenarios. The first being that the TDF were appropriate and the isotope plots showed a genuine dietary shift between individual geese. Secondly, the TDF were appropriate but the isotope plots indicated the use of endogenous reserves, incorporating unknown dietary sources. Thirdly, the TDF used were indeed inappropriate for the use of Canada goose primary feathers in this model, which can only be alleviated by conducting controlled feeding experiments.

Chapter Five
Conclusion

The use of seagrass (*Zostera muelleri*) habitat by Canada geese (*Branta canadensis*) in Waikato estuaries has been determined by a combination of three methods. Firstly, behavioural observations were conducted during the summer in Whāingaroa Harbour. Gut content analysis was then used to determine the food sources being ingested. Lastly, stable isotope analysis provided understanding in the contribution of *Zostera* as a food source to these geese, through assimilation into plasma, red blood cells, and feather tissues. The key findings of this study were:

- Canada geese allocated most of their time budget on the *Zostera* beds to foraging in summer. This was significantly influenced by the number of geese present and disturbance distance. Flocks of up to 200 birds were observed in January and February (summer), but geese were not present on *Zostera* beds in June (winter).

- Pasture grass is the predominant food source ingested by Canada geese during winter and spring. Although not observed on the *Zostera* beds during these months, isotope analyses of plasma and RBC tissues indicated that geese were still utilising a small proportion of *Zostera* and sea lettuce during this period.

- Pasture grass is the predominant food source assimilated into the plasma and red blood cells during winter and spring (87 to 93 %). Feather tissues of Canada geese also revealed pasture as the dominant food source during the summer moult, but was isotopically dispersed between pasture and another unknown dietary source. This variation may be due to the use of endogenous reserves or an inadequate trophic discrimination factor. *Zostera* and macroalgae made only a minor contribution to tissues during these periods. However, *Zostera* is the main contributing resource to black swans during the winter season, with a habitat shift to pasture grass during the summer moult period.

5.1 Future research and recommendations

There are several opportunities to develop the findings from this study. Firstly, due to the changes in Canada geese behaviours, habitats, and nutritional requirements throughout the year, it is possible they use different dietary sources across a temporal scale. Although behavioural observations in Whāingaroa identified that Canada geese feed on *Zostera* beds, this was not evident in their tissues. Sampling was temporally restricted due to Covid-19 lockdown. Therefore, collection of blood samples from mid-January (post-moult/end of summer) through to the end of March (beginning of autumn) is recommended to clarify the importance of *Zostera* in their diet. Separation of blood samples into plasma and red blood cells is not necessary, which will reduce processing time and costs. Furthermore, it is important to establish the exact time periods geese are utilising *Zostera* beds throughout the year. This could be a community led project to increase the number of observations. It would be beneficial to understand the disturbance geese cause to *Zostera* growth and reproduction, in light of the destructive foraging methods displayed by Canada geese in Whāingaroa Harbour and the response of the benthic community to the change in the structural components of *Zostera.* This could be conducted as a manipulative experiment, removing seagrass in a similar manner to foraging methods used by geese, and examining the response of macrofauna species. Although goose guano may not be a significant nutrient input in previously studied waterbodies (Mitchell & Wass, 1995), this may still be worth exploring in smaller, less well flushed estuaries known to have high bird densities. In conjunction with this the geese fouling effects *Zostera* primary productivity could also be assessed.

While this study cannot make specific suggestions on how to manage the Canada geese in Waikato estuaries, it is unlikely that numbers will decrease while geese remain under Schedule Five of the Wildlife Act 1953. Previous efforts to control population numbers including egg destruction, scare devices, toxic baits, moult culls, and recreational hunting (Spurr & Coleman, 2005), have not seen much long-term benefits (Spurr & Coleman, 2005). Without coordinated efforts between landowners, hunters, Fish & Game, the local council, and further research, the problems associated with Canada geese will likely continue to grow. Although this study focussed on the use of *Zostera* as a food source to Canada geese, isotope and gut content results revealed the importance of pasture grass as a food source. This reinforces some of the concerns farmers and landowners have raised to Waikato Regional Council regarding the prevalence of geese in the area and the need for catchment wide evaluation of their impacts. It is important

to conduct further research to understand not only the problems these geese pose to Waikato estuaries and the *Zostera* beds within them, but also on a catchment scale to capture the full extent of their disturbance potential.

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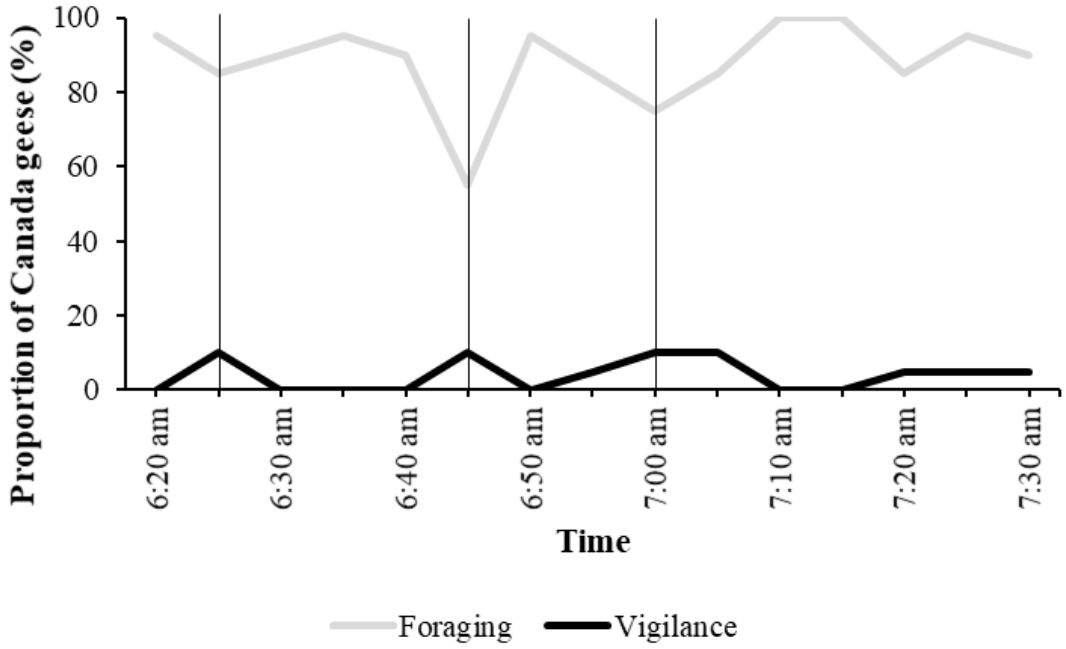
Appendix One

Behavioural data



Appendix Figure 1. 1. Graph showing the influence of disturbance events (black vertical line) on the proportion (%) of Canada geese foraging (grey horizontal line), and being vigilant (black horizontal line). Data was collected on the 30th January 2020 on *Zostera* beds at Cliff street (Site A).

Appendix Table 1. 1. Methods used by Canada geese (*Branta canadensis*) to forage on seagrass (*Zostera muelleri*) in Whāingaroa (Raglan) Harbour.

Behaviour	**Definition**
Upending	Geese swim above seagrass beds completely covered with water and reach down with their head underwater to forage.
Dabbling	Geese swim and/or stand above seagrass beds completely covered with water and paddle their feet rapidly in one location to stir up the sediment, then reach down with their heads underwater to grab the whole plant.
Wading	Geese walk through shallow water and ingest partially submerged seagrass.
Pecking	Geese walk and forage on exposed seagrass beds clipping blades.
Grubbing	Geese use their beaks to dig underneath exposed seagrass beds to grab the whole plant, including roots and rhizomes.
Trampling	Geese stomp their feet rapidly in one location to liquify the sediment and pull the whole plant out.

Appendix Two

Isotopic data

Appendix Table 2. 1. Comparison of the use of EDTA and SH anticoagulants on $\delta^{13}C$ isotope results of red blood cells (RBC) and plasma tissue samples.

	RBC (‰)		**Plasma (‰)**	
Bird no.	EDTA	SH	EDTA	SH
1	-28.73	-28.77	-28.69	-29.24
2	-29.62	-39.65	-29.84	-30.10
3	-27.85	-27.88	-28.27	-28.26
4	-28.29	-28.30	-28.13	-28.26
Mean (± SD)	-28.62 (± 0.76)	-31.15 (± 5.68)	-28.73 (± 0.78)	-28.97 (± 0.89)

Appendix Table 2. 2. Comparison of the use of EDTA and SH anticoagulants on $\delta^{15}N$ isotope results of red blood cells (RBC) and plasma tissue samples.

	RBC (‰)		**Plasma (‰)**	
Bird no.	EDTA	SH	EDTA	SH
1	4.91	4.93	4.60	6.33
2	5.14	5.20	6.01	6.63
3	5.85	5.93	6.87	7.27
4	5.52	5.52	5.73	6.53
Mean (± SD)	5.36 (± 0.41)	5.40 (± 0.43)	5.80 (± 0.94)	6.69 (± 0.41)

Appendix Table 2. 3. Discrimination source file showing the trophic discrimination factors estimated by SIDER (Healy et al., 2018) for Canada geese feather, plasma, and red blood cell (RBC) tissues.

	Δ δ^{13}C (± SD)	**Δ δ^{15}N (± SD)**
Feather	0.56 (± 1.35)	5.68 (± 1.58)
Blood (plasma and RBC)	-0.50 (± 1.31)	4.75 (± 1.56)

Appendix Table 2. 4. Food source file showing the Mean isotopic signatures, concentrations, and number of samples, of each food source (pasture grass, *Zostera muelleri*, macroalgae, and Yellow-bellied flounder (*Rhombosolea leporina*)).

	Mean δ^{13}C (± SD)	**Conc. δ^{13}C**	**Mean δ^{15}N (± SD)**	**Conc. δ^{15}N**	**n**
Pasture	-27.99 (± 3.09)	42.09	3.00 (± 3.05)	2.75	15
Zostera	-11.29 (± 0.90)	34.51	6.05 (± 0.66)	1.66	16
Macroalgae	-18.78 (± 0.64)	35.66	7.36 (± 0.40)	1.69	5
Flounder	-16.86 (± 1.57)	45.65	13.01 (± 0.77)	14.10	6

Appendix Table 2. 5. Consumer source file of the isotopic values from black swan (*Cygnus atratus*) and Canada goose (*Branta canadensis*) plasma tissues.

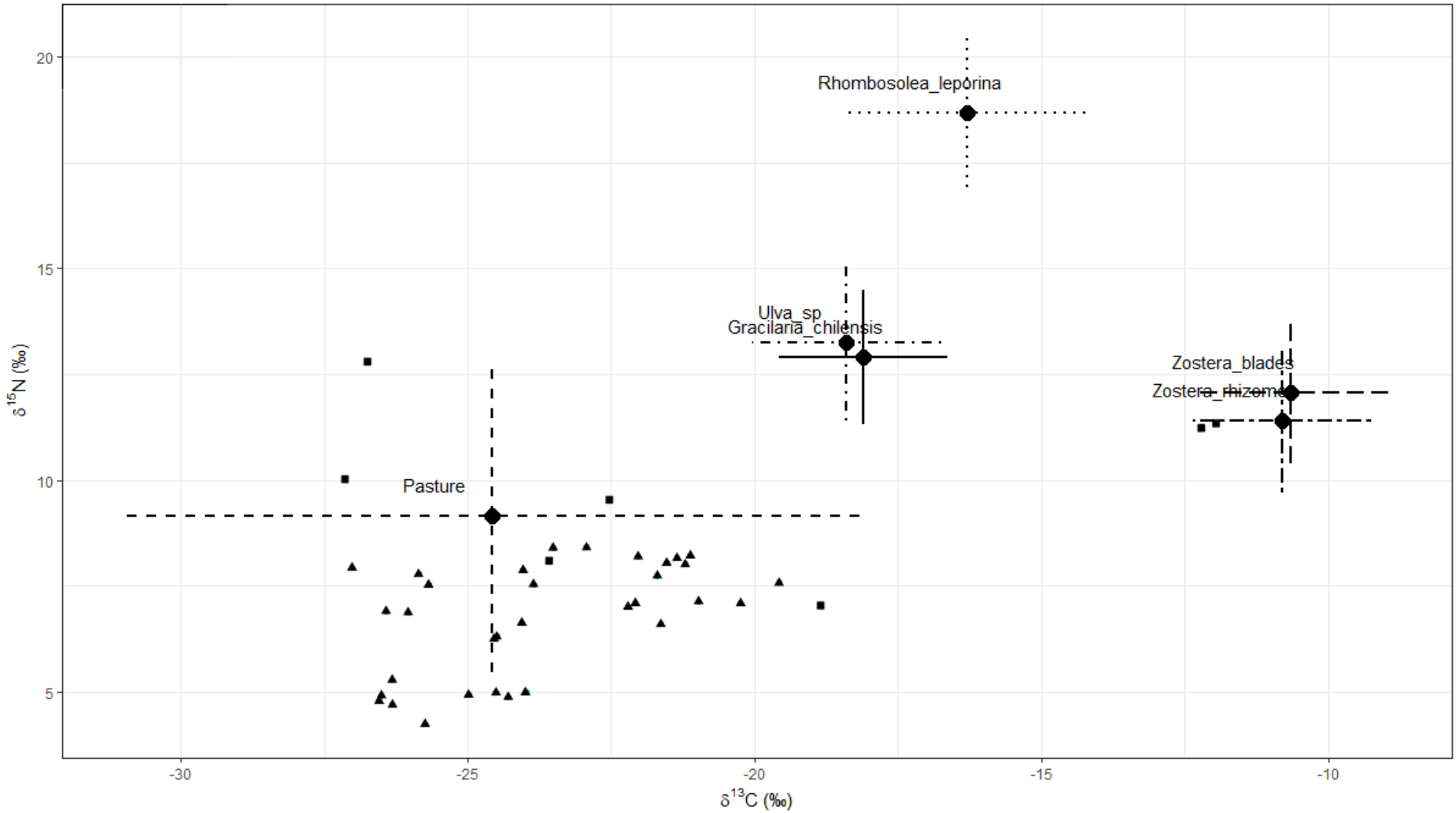
Black swan		Kawhia geese		Raglan geese	
$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$
8.62	-27.24	5.92	-28.95	6.47	-28.67
8.71	-27.07	5.37	-28.51	7.32	-28.84
9.19	-12.36	6.22	-29.77	6.12	-28.47
9.96	-11.21	5.93	-28.56	7.34	-29.40
9.43	-12.27	7.17	-28.22	6.69	-28.83
10.87	-15.80	6.25	-29.64	6.48	-29.29
9.66	-11.89	6.10	-28.11	6.41	-29.61
9.81	-11.94	6.62	-28.24	6.12	-29.52
10.22	-18.26	7.22	-28.28	6.48	-29.42
11.06	-11.63	7.15	-26.86	6.69	-29.56
9.54	-13.15	7.86	-25.28	6.61	-29.32
		6.83	-26.22	6.19	-29.38
		6.95	-29.09	6.21	-29.26
		7.46	-28.03	5.76	-29.26
		6.21	-29.62	6.05	-29.24
		7.99	-28.77	7.05	-30.00
		7.28	-28.56	6.17	-29.24
		7.70	-28.76	6.61	-29.72
		7.86	-28.04	6.15	-29.75
		8.39	-28.20	6.16	-29.53
		7.80	-28.33	6.38	-29.70
		6.50	-28.58	5.82	-29.23
		7.49	-28.90	7.12	-29.86
		7.58	-28.79	7.09	-29.57
		5.79	-27.95	6.33	-29.66
		7.90	-28.54	7.02	-30.19
		7.90	-28.40		
		5.89	-27.38		
		5.97	-27.58		
		4.93	-27.35		
		5.60	-27.47		
		5.60	-27.28		
		5.29	-26.88		
		7.25	-25.85		
		4.27	-25.02		

Appendix Table 2. 6. Consumer source file of the isotopic values from black swan (*Cygnus atratus*) and Canada goose (*Branta canadensis*) red blood cell (RBC) tissues.

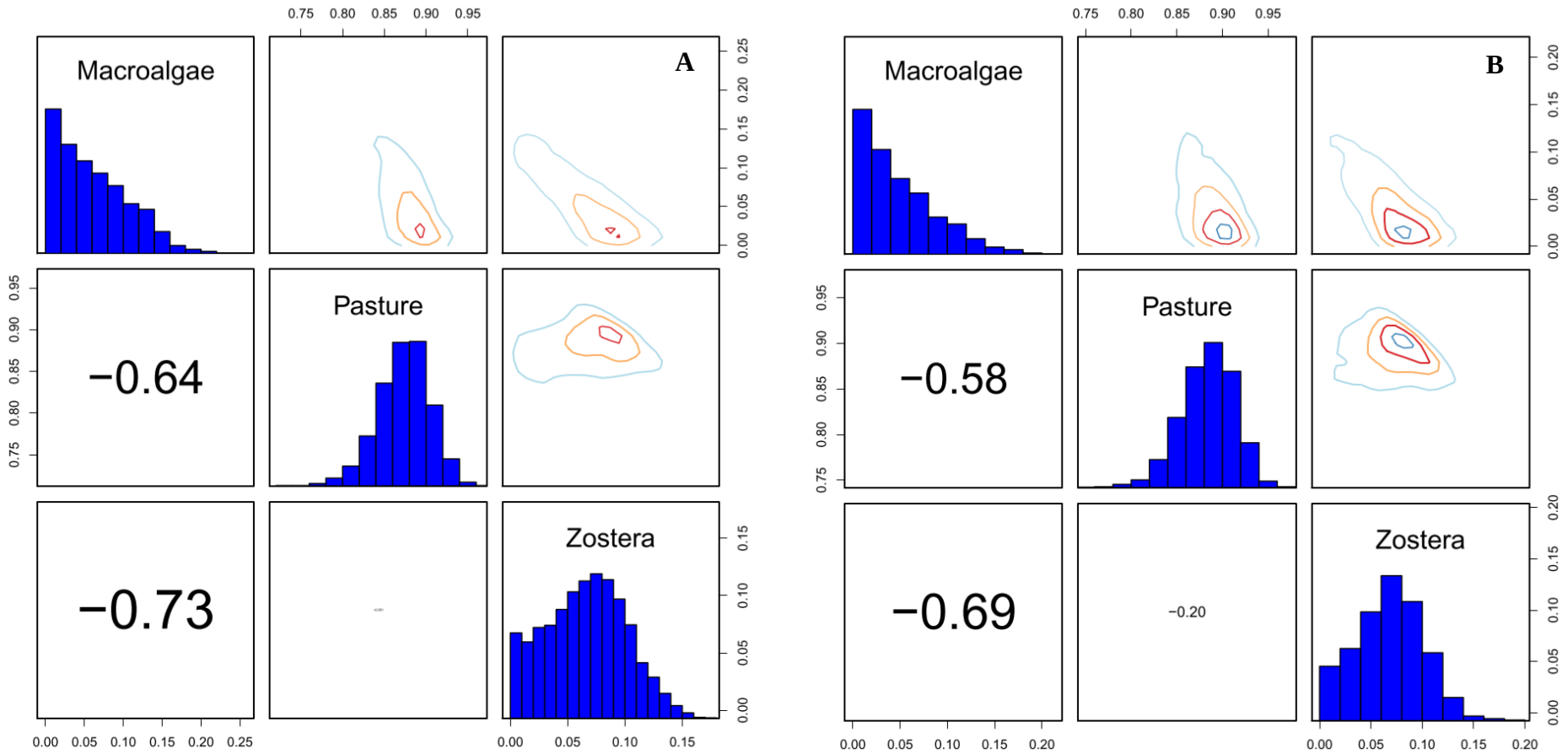
Black swan		Kawhia geese		Raglan geese	
$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$
8.60	-25.68	5.48	-29.62	6.16	-29.85
8.26	-24.70	5.05	-29.57	5.75	-29.74
8.58	-16.75	6.70	-28.94	6.13	-29.73
9.65	-11.62	5.40	-28.80	6.24	-29.73
9.36	-11.64	4.94	-28.74	5.98	-29.71
9.69	-15.32	5.39	-28.71	6.18	-29.69
9.29	-13.09	7.60	-28.67	6.13	-29.63
9.79	-12.51	6.89	-28.61	5.68	-29.62
11.08	-24.76	7.13	-28.59	6.05	-29.57
10.78	-10.80	6.21	-28.59	6.12	-29.57
9.14	-14.95	7.10	-28.53	4.93	-29.56
		4.21	-28.49	6.37	-29.55
		7.11	-28.40	6.06	-29.55
		7.05	-28.40	5.52	-29.49
		7.81	-28.36	5.94	-29.43
		6.37	-28.36	5.60	-29.37
		7.28	-28.32	6.55	-29.35
		6.61	-28.30	5.15	-29.30
		5.68	-28.30	5.08	-29.21
		6.87	-28.30	5.52	-29.11
		5.74	-28.28	5.71	-28.98
		7.15	-28.03	6.80	-28.80
		6.03	-27.85	6.32	-28.79
		5.16	-27.74	6.19	-28.67
		5.27	-27.72	6.06	-28.61
		5.40	-27.68	6.11	-28.30
		5.43	-27.64		
		6.68	-27.52		
		5.14	-27.08		
		6.10	-26.95		
		6.11	-26.91		
		6.19	-26.19		
		4.44	-25.76		
		6.32	-24.89		
		3.45	-24.76		

Appendix Table 2. 7. Consumer source file of the isotopic values from black swan (*Cygnus atratus*) and Canada goose (*Branta canadensis*) feather tissues.

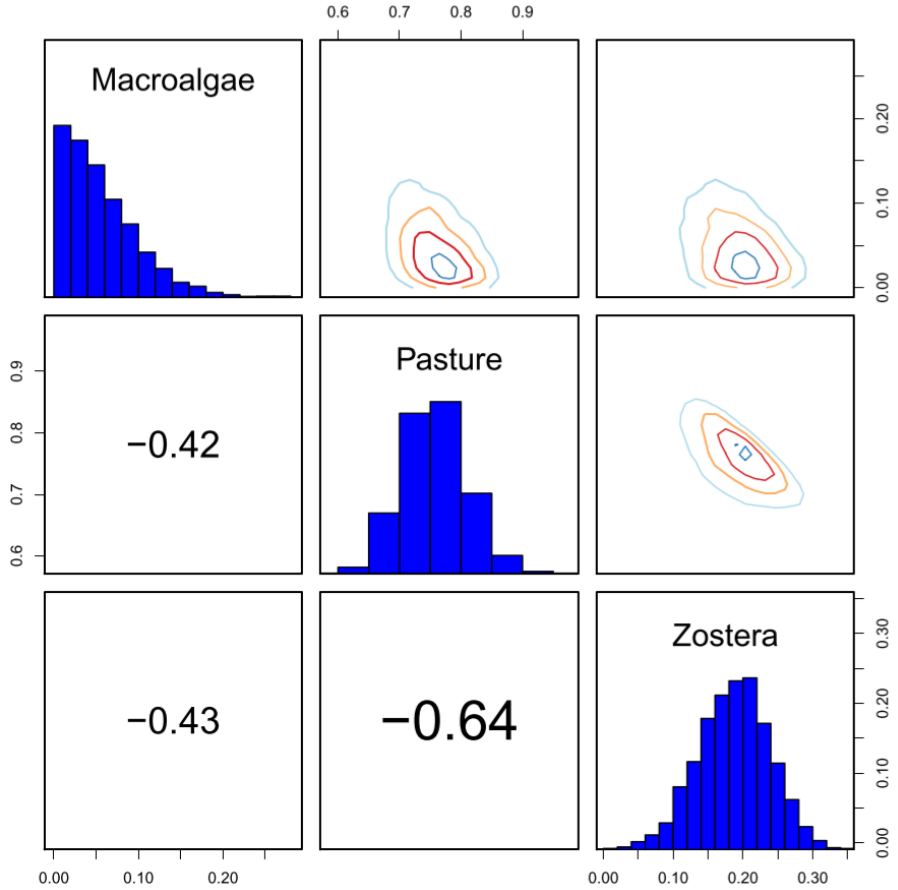
Black swan		Kawhia geese		Raglan geese	
$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$
10.06	-27.14	7.94	-27.02	9.37	-26.55
12.83	-26.77	4.78	-26.55	9.30	-26.48
8.12	-23.58	4.91	-26.51	5.89	-26.46
9.53	-22.52	6.91	-26.42	4.90	-25.99
7.05	-18.85	5.29	-26.32	7.31	-25.98
11.27	-12.21	4.71	-26.32	5.29	-25.85
11.35	-11.94	6.86	-26.05	5.51	-25.82
		7.80	-25.86	7.48	-25.80
		4.23	-25.75	6.64	-25.73
		7.53	-25.69	6.21	-25.69
		6.21	-25.37	8.98	-25.68
		4.93	-24.97	6.92	-25.67
		6.27	-24.54	8.63	-25.57
		4.98	-24.52	7.69	-25.43
		6.32	-24.48	7.25	-25.21
		4.87	-24.29	8.71	-24.99
		6.63	-24.07	5.54	-24.92
		7.88	-24.04	6.68	-24.85
		4.99	-23.98	7.08	-24.17
		7.52	-23.86	7.86	-24.07
		6.23	-23.71	8.03	-23.83
		6.14	-23.59	8.82	-23.37
		8.41	-23.50	8.42	-23.11
		6.59	-23.11	8.13	-21.68
		8.42	-22.93	11.13	-19.70
		7.00	-22.21		
		7.09	-22.08		
		8.21	-22.05		
		7.74	-21.69		
		6.62	-21.63		
		8.04	-21.52		
		8.17	-21.37		
		8.02	-21.20		
		8.23	-21.13		
		7.13	-20.97		
		7.10	-20.25		
		7.59	-19.58		



Appendix Figure 2. 1. Isospace plot of the isotopic signatures from Canada geese and black swan feathers (representing moulting/feather regrowth diet). Error bars show the standard deviation of the mean (●) of each food source, including flounder (*Rhombosolea leporina*), which have been adjusted with TDF. Consumer information is displayed for male black swans (■), and Canada geese (▲)



Appendix Figure 2. 2. Matrix plot of the posterior dietary proportions obtained from the Canada geese plasma (A) and red blood cells (B) data. The upper diagonal shows contour plots; the diagonal, histograms; and the lower-diagonal, the correlations between the different food sources.



Appendix Figure 2. 3. Matrix plot of the posterior dietary proportions obtained from the Canada geese feather data. The upper diagonal shows contour plots; the diagonal, histograms; and the lower-diagonal, the correlations between the different food sources.

Appendix Table 2. 8. Gelman-Rubin diagnostics for stable isotope mixing models plasma, red blood cells (RBC), and feather tissues. This should be <1.05.

	> 1.01	**> 1.05**	**> 1.10**
Plasma (n = 91)	0	0	0
RBC (n = 91)	0	0	0
Feather (n = 88)	0	0	0

Appendix Table 2. 9. Geweke diagnostics for stable isotope mixing models of plasma, red blood cells (RBC), and feather tissues. This is a standard z-score, so <5% of the number of variables (n) are expected to be outside ± 1.96 in each chain.

	Chain 1	**Chain 2**	**Chain 3**
Plasma (n = 91)	1	0	0
RBC (n = 91)	0	0	0
Feather (n = 88)	1	0	0

Appendix Table 2. 10. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) trophic discrimination factors from previous studies for blood (whole), red blood cells (RBC), plasma, and feather tissues of different avian species.

Tissue	Foraging guild	Species	Scientific name	Diet	Δ $\delta^{13}C$	Δ $\delta^{15}N$	Reference
RBC	herbivore	Bewick's swan	*Cygnus columbianus bewickii*	Grain + chicken mash	-0.69	3.69	(Hahn et al., 2012)
Plasma	herbivore	Bewick's swan	*Cygnus columbianus bewickii*	Grain + chicken mash	0.42	4.04	(Hahn et al., 2012)
Feather	herbivore	Bewick's swan	*Cygnus columbianus bewickii*	Grain + chicken mash	0.64	5.39	(Hahn et al., 2012)
Plasma	insectivore	Dunlin	*Calidris alpina*	Wheat/animal protein mix	0.5	3.3	(Ogden et al., 2004)
RBC	insectivore	Dunlin	*Calidris alpina*	Wheat/animal protein mix	1.5	3.0	(Ogden et al., 2004)
Blood	insectivore	Garden Warbler	*Sylvia borin*	Fruit + insects	1.7	2.4	(Hobson & Bairlein, 2003)
Feather	insectivore	Garden Warbler	*Sylvia borin*	Fruit + insects	2.7	4.0	(Hobson & Bairlein, 2003)
Blood	carnivore	Great Skua	*Catharacta skua*	Fish (incl. Lipid)	4.3	2.6	(Bearhop et al., 2002)
Blood	carnivore	Great Skua	*Catharacta skua*	Fish (excl. Lipid)	1.1	2.8	(Bearhop et al., 2002)
Blood	carnivore	Great Skua	*Catharacta skua*	Beef (incl. Lipid)†	7.1	4.0	(Bearhop et al., 2002)
Blood	carnivore	Great Skua	*Catharacta skua*	Beef (excl. Lipid)†	2.3	4.2	(Bearhop et al., 2002)
Feather	carnivore	Great Skua	*Catharacta skua*	Fish (incl. Lipid)	5.3	4.4	(Bearhop et al., 2002)
Feather	carnivore	Great Skua	*Catharacta skua*	Fish (excl. Lipid)	2.1	4.6	(Bearhop et al., 2002)

Tissue	Foraging guild	Species	Scientific Name	Diet	Δ $\delta^{13}C$	Δ $\delta^{15}N$	Reference
Feather	carnivore	Great Skua	*Catharacta skua*	Beef (incl. Lipid)†	7.0	4.8	(Bearhop et al., 2002)
Feather	carnivore	Great Skua	*Catharacta skua*	Beef (excl. Lipid)†	2.2	5.0	(Bearhop et al., 2002)
Blood	granivore	Japanese Quail	*Coturnix japonica*	Turkey starter	1.2	2.2	(Hobson & Clark, 1992b)
Feather	granivore	Japanese Quail	*Coturnix japonica*	Turkey starter	1.4	1.6	(Hobson & Clark, 1992b)
Blood	piscivore	King Penguin	*Aptenodytes patagonicus*	Whole fish	-0.81	2.07	(Cherel et al., 2005)
Blood	piscivore	King Penguin	*Aptenodytes patagonicus*	Fish muscle	-0.61	1.23	(Cherel et al., 2005)
Feather	piscivore	King Penguin	*Aptenodytes patagonicus*	Whole fish	0.07	3.49	(Cherel et al., 2005)
Feather	piscivore	King Penguin	*Aptenodytes patagonicus*	Fish muscle	0.26	2.65	(Cherel et al., 2005)
RBC	herbivore	Mallard	*Anas platyrhynchos*	Grain + chicken mash	-0.34	3.6	(Hahn et al., 2012)
Plasma	herbivore	Mallard	*Anas platyrhynchos*	Grain + chicken mash	0.18	4.67	(Hahn et al., 2012)
Feather	herbivore	Mallard	*Anas platyrhynchos*	Grain + chicken mash	1.12	5.11	(Hahn et al., 2012)
Blood	carnivore	Peregrine Falcon	*Falco peregrinus*	Quail muscle	0.2	3.3	(Hobson & Clark, 1992b)
Feather	carnivore	Peregrine Falcon	*Falco peregrinus*	Quail muscle	2.1	2.7	(Hobson & Clark, 1992b)
Blood	piscivore	Ringbilled Gull	*Larus delawarensis*	Fish (perch)	-0.3	3.1	(Hobson & Clark, 1992b)

Tissue	Foraging guild	Species	Scientific Name	Diet	Δ $\delta^{13}C$	Δ $\delta^{15}N$	Reference
Feather	piscivore	Ringbilled Gull	*Larus delawarensis*	Fish (perch)	0.2	3	(Hobson & Clark, 1992b)
Blood	piscivore	Rockhopper Penguin	*Eudyptes chrysocome*	Whole fish	0.02	2.72	(Cherel et al., 2005)
Blood	piscivore	Rockhopper Penguin	*Eudyptes chrysocome*	Fish muscle	0.46	1.86	(Cherel et al., 2005)
Feather	piscivore	Rockhopper Penguin	*Eudyptes chrysocome*	Whole Fish	0.11	4.40	(Cherel et al., 2005)
Feather	piscivore	Rockhopper Penguin	*Eudyptes chrysocome*	Fish muscle	0.55	3.53	(Cherel et al., 2005)