New Zealand Petrel Translocation Diets

An assessment of three Procellariiformes diets: grey-faced petrel (Pterodroma macroptera gouldi; kuia), Chatham petrel (Pterodroma axillaris; ranguru) and fluttering shearwater (Puffinus gavia; pakahā).

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Grey-faced petrel chick

Pterodroma macroptera

Abstract

In New Zealand, a pureed diet of Brunswick tinned sardines™ in soya oil (S/Soy) has been widely fed to Procellariiformes chicks during translocations. The diet provides high fledging rates, however, when fed for longer periods diseases have occurred that may be related to malnutrition and may impact fledgling survival at sea. The effect of dietary oils was compared in feeding trials by substituting soya oil with fish oil during chick translocations of two burrow nesting petrel species. Fish oil supplemented diets were fed to 30 of 76 grey-faced petrel chicks (*Pterodroma macroptera gouldi;* kuia) (GFP) combined with a Mazuri fish analogue® (M/Fish), and to 20 of 74 fluttering shearwater chicks (*Puffinus gavia*; pakahā) (FS) fed a puree of Brunswick tinned sardines™ in spring water (S/Fish). Changes in red blood cell (RBC) phospholipid fatty acid composition, fledging parameters, and necropsy results were compared over the 3-week translocation period as well as return rates thereafter. Fledging parameters were similar between the diet groups, with GFP chicks that were fed the S/Soy diet fledging at significantly higher weights. Deaths due to visceral gout occurred in GFP chicks irrespective of the diet fed. Feeding fish oil improved fledging RBC phospholipid fatty acid ratios to levels that resemble wild seabird diets, with significantly higher docosahexaenoic acid C22:6n3 (DHA) and arachidonic acid C20:4n6 (ARAC); fatty acids that are important for cellular communication as eicosanoids and are vital for immune responses in birds. In contrast, chicks fed soya oil had significantly higher proportions of linoleic acid C18:2n6 (LIN) resulting in RBC membranes that were filled with plant-based 18-carbon fatty acids, which may not be metabolizable in some obligate piscivorous seabirds. DHA levels were significantly decreased which could have a deleterious effect on chick maturation. Until the exact nutritional requirements of seabirds are known a prudent diet would include fish oils that are already present in wild marine-based diets. Return rates of FS nine years later have not shown any difference in effect from the oil-fed.

The three species of petrel were investigated to represent three, distinctly different foraging strategies: GFP as long-distance foragers, FS as short-distance daily feeders and Chatham petrel (*Pterodroma axillaris*; ranguru) (CP) as an endangered species that are long-distance foragers, yet have a restricted range during the chick-rearing season. The nutrient composition and fatty acid components of proventricular samples for each species were analysed and compared to three different translocation dietary groups (S/Soy, M/Fish and S/Fish). The effect of sampling methods on nutrient analysis was compared between proventricular flushing (PVF) and spontaneous regurgitation (REG).

Results showed that GFP diets were highly variable in nutritional composition and the sampling method had a significant effect on results, with REG samples demonstrating higher fat content. Ash content was highly variable in all species, particularly samples collected by PVF, and increased the variation reported in proximate analysis results. Yet, irrespective of the sampling method used, the differences between all species were widely apparent. Species with a short-foraging strategy (FS) show higher protein and lower fat content compared with those with long-foraging strategies (GFP and CP). The fatty acid proportions of PVF samples were not greatly affected by sampling methods but showed diversity when compared between species and translocation dietary groups. The artificial diet S/Soy had significantly higher proportions of LIN and alpha-linolenic acid C18:3n3 (ALIN) than any other group, setting it apart distinctly from all other wild diets as well as artificial diets supplemented with fish oil. DHA and ARAC were lower in the soya oil diet than in both the fish oil supplemented diets and in the GFP wild diet. Oleic acid C18:1n9 (OLE) and palmitic acid C16:0 (PAL) were the predominant fatty acids in wild diets and showed species-specific differences. No artificial diet provided sufficient nervonic acid C24:1n9 (NERV) to reach levels in wild diets, with tinned sardines in fish oil being the closest alternative. The CP wild diet was uniquely high in NERV and its n-9 precursors (OLE, eicosenoic acid C20:1n9 and erucic acid C22:1n9), with markedly low DHA, docosapentaenoic acid C22:5n3 (DPA), eicosapentaenoic acid C20:5n3 (EPA), and ARAC levels in comparison to other species, wild diets, and translocation dietary groups. Translocation diets based on tinned sardines with supplemented fish oil showed closest similarity to the wild diets of all species studied. Given the dietary importance of long-chain polyunsaturated fatty acids (PUFAs) for the health and development of chicks, a prudent diet would include supplementation with DHA, ARAC and NERV fortified oils. The volume and proportion of fat-fed in the diet needs further investigation, with careful consideration of the sampling methods used to determine normal fat levels within the diet.

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ALIN	C18:3n3 - cis-9,12,15-Alpha linolenic acid
ARAC	C20:4n6 - cis-5,8,11,14-Arachidonic acid
CAP	C6:0 Caproic acid
CP	Chatham petrel *Pterodroma axillaris, (*ranguru)
DHA	C22:6n3 - cis-4,7,10,13,16,19 Docosahexaenoic acid
DOC	New Zealand Department of Conservation.
DPA	C22:5n3 – cis-7,10,13,16,19 Docosapentaenoic acid
EPA	C20:5n3 – cis-5,8,11,14,17- Eicosapentaenoic acid
FS	Fluttering shearwater *Puffinus gavia,* (pakahā)
GFP	Grey-faced petrel *Pterodroma macroptera gouldi,* (kuia)
GLM	General linear model
LIN	C18:2n6c Linoleic acid
Mid-transfer	Blood samples were collected as close to the transfer from the wild as possible, 6-10 days in grey-faced petrels and 3-4 days in fluttering shearwaters.
M/Fish	Mazuri fish analogue diet® with fish oil added
NERV	C24:1n9 - cis-15- Nervonic acid
OLE	C18:1n9c Oleic acid
PAL	C16:0 Palmitic acid
PCA	Principal component analysis
PERMANOVA	Permutational multivariate analysis of variance
Pre-fledge	Blood samples were taken at the mid-transfer blood sample as close to chick fledging dates as could be predicted, 14-18 days in grey-faced petrels and 13-14 days in fluttering shearwaters.
PUFA	Polyunsaturated fatty acids
PVF	Proventricular flushing, collecting wild diet samples by flushing saline into the proventriculus.
RBC	Red blood cells /erythrocytes.
REG	Samples collected by spontaneous regurgitation
SAM	Wildlife clinic diet of frozen salmon slurry with multivitamins added and no additional oil.
S/Fish	Brunswick tinned sardines in spring water™, drained, with fish oil added
S/Soy	Brunswick tinned sardines in soya oil™
Wild	Wild diet of grey-faced petrel chicks from a wild control colony at Te Henga.

Glossary of terms

Eicosanoid	Any of a class of compounds (such as the prostaglandins) derived from polyunsaturated fatty acids and involved in cellular activity.
Foraging strategy	The particular method for locating food, whether they smell it, find it by sight or detect it by chemical means.
Phospholipid membrane	Phospholipids are major components of biological cell membranes and are responsible for dynamic membrane fluctuations and cell signalling. Phospholipids typically consist of a phosphate head and a tail of two fatty acids, which form the lipid bilayer membrane of cells, including red blood cells.
Polyunsaturated fatty acid (PUFA)	A fatty acid that contains more than one double bond (C=C). The essential fatty acids, omega-3, omega-6 and omega-9's are polyunsaturated fatty acids (PUFAs)
Proventriculus	The glandular part of Procellariiformes birds gastrointestinal tract situated between the crop and ventriculus (gizzard).
Proventriculitis/ventriculitis	Inflammation of the proventriculus or ventriculus of a bird.
Proximate analysis	Quantitative analysis of a mixture of food to determine the percentage of components.
Very long-chain PUFA	Polyunsaturated fatty acids with 20 carbons or more.

Chapter One

1 Introduction to petrel translocation diets in New Zealand

1.1 Conservation benefits of seabird colony translocation.

Translocation of burrow-nesting seabird chicks is a pivotal conservation tool used to protect and propagate colonies of petrels in New Zealand (Gummer, 2003). The need for intensive management of wild petrel populations is becoming more critical, as globally 29% of all seabird species globally are threatened with extinction (Borrelle et al., 2018). Within New Zealand alone there are at least 36 species of burrow-nesting and surface-nesting petrels. Of these: 33 species suffer from human-induced reductions in breeding range, and a further six are nationally critical or nationally endangered (Miskelly et al., 2009).

Translocations aid seabird recovery programmes by increasing the distribution of threatened species to areas that are free of predation, habitat destruction, and human disturbance (Gummer H 2003). New colonies provide a safeguard of additional breeding sites for species that are vulnerable to threats in their present colonies. (Gummer 2003). Translocations are also used to re-colonise historic sites where local extinction has occurred or supplement numbers of individuals in newly seeded colonies (Jones & Kress, 2012). Colonies of burrow-nesting seabirds also act as ecological drivers for the restoration of disturbed habitats via the importation of vast quantities of marine nutrients (especially nitrates and phosphates), and the creation of a subterranean network of tunnels that provide habitat to other species (Miskelly, 2006). Techniques developed to embed new colonies may also be used to support recovery from invasive species, unexpected ecological disasters due to inclement weather events, and human impacts such as oil spills (Jones & Kress, 2012).

There are few known examples of petrels naturally recolonising sites following their total extirpation, mainly due to their strong homing instinct (philopatry) to return to their natal site and because their population growth is very slow (maximum of one chick per pair per year) (Miskelly, 2006; Miskelly & Taylor, 2004; Warham, 1990). Few populations are increasing rapidly enough to seek new colonies or re-colonise vacated breeding grounds without human intervention. (Miskelly, Taylor et al. 2009).

1.1.1 Methods of chick translocations

Colony translocation involves moving chicks from their natal burrow to a different colony site, where the chicks are hand-raised in artificial burrows. New colony site imprinting is likely to occur when the chicks emerge from their burrow and fledge. Translocated chicks return as adults to reproduce and will also attract pre-breeding birds from other colony sites. With the aid of acoustic signalling and a range of social attraction methods, translocation has proven to be effective for the formation of new colonies, (Gummer, Taylor, Collen, et al., 2014).

Translocation is likely to be the only effective way of starting a new colony in a location that is far away from the usual flight path of a species (Gummer, Taylor, Collen, et al., 2014). Techniques are constantly evolving, especially concerning artificial diets. (Gummer, Taylor, Collen, et al., 2014).

1.1.2 Translocation diets

The central aspect of translocations is that chicks must be moved within five weeks prior to fledging so that they will site imprint at the new location. This entails hand-rearing chicks in often remote locations.

New Zealand has provided many important studies for seabird translocation and restoration projects (Jones & Kress, 2012) and over time the translocation diet has improved as feeding techniques have evolved. In the 1990s, chicks were fed a pureed diet of pilchards, salmon, squid or krill, with various oils added (from vegetable oil to mutton bird oil) (Bell et al., 2005; Miskelly et al., 2009), however preparation and storage of these diets were often difficult in remote field sites (Taylor et al., 2021). Although this diet was capable of producing good fledging rates in some years, frozen seafood diets were also prone to bacterial contamination, which led at times to higher death rates. (Bell et al., 2005; Taylor et al., 2021).

Chick fledging rates improved during translocations as the standards of food preparation advanced, and the translocation diet was transitioned to a diet consisting of Brunswick tinned sardines in soya oil™ (juvenile Antarctic herrings *Clupea harengus),* with Mazuri® Vita-Zu® multi-vitamins added (S/Soy) (see Table 1) (Miskelly et al., 2009; Taylor et al., 2021). By 2009, 1,454 chicks has been translocated and hand-reared on the S/Soy diet (Miskelly et al., 2009). Nine species of petrels and shearwaters[1] have been fed the S/Soy diet (Gummer, Taylor, et al., 2014a; Miskelly et al., 2009; Taylor et al., 2021), irrespective of their different foraging strategies and potentially diverse prey items. The S/Soy diet was the first diet fed to critically endangered Chatham Island (*Magenta petrel; Pterodroma magenta*; tāiko) chicks. Yet despite its widespread use, the S/Soy diet has never been nutritionally assessed for hand-raising seabirds. The New Zealand Department of Conservation (DOC) protocols do not recommend feeding the hand-rearing diet for more than 4-5 weeks (Gummer, Taylor, Collen, et al., 2014) as historically there have been higher deaths related to nutritional disease (McInnes, 2007).

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[1] Common diving petrel (*Pelecanoides urinatrix*; kuaka), fairy prion (*Pachyptila turtur;* tītī wainui), grey-faced petrel (*Pterodroma macroptera gouldi*; kuia), Pycroft's petrel (*Pterodroma pycrofti*), Chatham petrel (*Pterodroma axillaris*; ranguru), Chatham Island taiko (*Magenta petrel; Pterodroma magenta*; tāiko), fluttering shearwater (*Puffinus gavia*; pakahā), and Hutton's shearwater (*Puffinus huttoni*; Kaikōura tītī) and Cook's petrel (*Pterodroma cookie; tītī*)

Previous grey-faced petrel (*Pterodroma macroptera gouldi*; kuia) (GFP) translocations have reported sporadic deaths due to proventriculitis, ventriculitis, visceral gout, renal compromise, cloacal impaction and hepatic lipidosis (McInnes, 2007). This raises the concern that birds may be fledging with sub-clinical levels of nutritional disease that could impact their survival at sea.

This study assesses the nutritional composition of the S/Soy translocation diet by comparing it to the proventricular content of wild seabirds of several species and to an alternative powdered Mazuri® seabird diet. By health screening petrels during translocation and undertaking dietary trials, the potential contribution of translocation diets to health concerns before fledging was assessed. Proventricular samples from three species of Procellariidae chicks were compared to assess sampling methods and nutritional composition when creating an artificial diet.

1.2 Importance of oils in Procellariiformes diets

1.2.1 Proventricular oil

Proventricular oils are incredibly important to burrow nesting seabirds diets. The Procellariiformes (albatrosses, shearwaters, and petrels) are unique among birds as they store large quantities of oil in the proventriculus, a glandular stomach that separates the oesophagus from the gizzard (Warham et al., 1976). Dietary lipids are the most concentrated source of food a bird can consume and also supply fatty acids and pigments. (Klasing, 1998). The oils are accumulated in the proventriculus by delayed gastric emptying where the oil fraction of partially digested prey (chyme) is concentrated and retained longer than the aqueous fraction (Foster et al., 2010; Roby et al., 1989). Compared to the energy density of the original prey items they consume, the energy density of the concentrated oil can increase from a factor of 5 to 35. The high energy oil can then easily be transported by the birds while foraging to provision their chicks (Foster et al., 2010; Warham, 1977; Warham et al., 1976).

There are many beneficial functions of proventricular oil. Some species of petrel can regurgitate stomach oils as a defence mechanism (Padilla, 2015) or offload oils as they are lightening their weight in preparation for fledging (Baduini & Hyrenbach, 2003). Olfactory cues from the diet fed to nesting chicks have been linked to learned foraging behaviours and nest recognition in Procellariiformes. (Cunningham & Nevitt, 2011; Cunningham et al., 2003; Nevitt et al., 1995; Van Buskirk & Nevitt, 2008). Dietary fat plays an important role in the function of the uropygial gland (preen gland), the primary role of which is lipogenesis, secreting sebum to maintain feather pliability and waterproofing with additional roles in pheromone secretion, as well as antibacterial and antimicrobial functions. (McWilliams, 2008)

1.2.2 The importance of dietary oils

Dietary fatty acids are incorporated into cell membranes as phospholipids forming the essential structural support for the cell membranes lipid bilayer (Cherian, 2015). The fatty acid composition of phospholipid membranes in immune cells can affect the magnitude of an inflammatory response in all mammals (Korver & Klasing, 1997). Very long-chain polyunsaturated fatty acids (PUFA) improve membrane fluidity and cell communication as the precursors of eicosanoids (inflammatory mediators) (Harrison & Lightfoot, 2006; Klasing, 1998). During chick growth early exposure to PUFAs, docosahexaenoic acid C22:6n3 (DHA) and arachidonic acid C20:4n6 (ARAC), is crucial for the optimal functioning of cells, immunoregulation and the development of brain, retina, skeletal and cardiac tissues (Cherian, 2015; Koppenol et al., 2014). In poultry, adding fish oil to the diet has been shown to have positive effects on growth and lessoned the growth-suppressing effect of disease exposure by improving the immune responses. (Korver & Klasing, 1997).

1.2.3 Omega 3 and omega 6 polyunsaturated fatty acids.

The omega-3 and omega-6 PUFA groups are known for their wide array of health benefits due to their important role as inflammatory mediators and lipid stabilizing qualities (Harrison & Lightfoot, 2006). The omega groups are named after the position of the first double bond from the ‘omega’ or terminal end of the fatty acid molecule. The backbone of PUFA is a very long polyunsaturated carbon chain, with more than 20 carbons (>C20). The omega-3 fatty acids include DHA, eicosapentaenoic acid C20:5n3 (EPA), and docosapentaenoic acid C22:5 n3 (DPA) with the precursor 18 carbon chain fatty acid α-linolenic acid C18:3 n-3 (ALIN). The omega-6 fatty acids include arachidonic acid C20:4 n-6 (ARAC) and the precursor molecule linoleic acid C18:2 n-6 (LIN) (Bradbury, 2011).

1.2.4 Fatty acid synthesis

DHA and EPA are synthesized in abundance by marine algae and found concentrated in fish and marine oils (Bradbury, 2011). Fish oil and proventricular oil are both naturally rich in very long-chain PUFA, especially omega-3 fatty acids and omega-6 fatty acids. Plant-based oil, such as soya oil contains PUFA that have shorter 18 carbon chains, ALIN and LIN, which when consumed in the diet act as building blocks that are elongated and desaturated into very long-chain PUFA. Soya oil is very high in LIN and ALIN. Species of birds that eat only marine-based organisms may have a requirement for already formed very long-chain PUFA (ARAC and DHA) (Klasing, 1998; Speer, 2015). During evolution, many strictly carnivorous or piscivorous species have lost the need and ability to elongate and desaturate C18 fatty acids because their wild diets are already naturally rich in the end products of this transformation, (i.e. very long-chain

PUFA, ARAC, DHA and EPA) (Klasing, 1998). Even in avian species where DHA synthesis is possible (such as poultry), fish oil supplementation is still recommended because it results in higher brain DHA concentration (Cherian, 2015) and reduces the competition for enzymes that synthesize DHA (Koppenol et al., 2014).

The value of the kind of fatty acids that are added to an artificial diet is dependent on how well the fatty acids are utilized. There is sparse information in the literature about the capability of Procellariiformes seabirds to synthesize fatty acids from LIN and ALIN, as the end products of these transformations are already abundantly found in the natural diet (Klasing, 1998).

A common finding in birds generally is that monosaturated fatty acids oleic acid 18:1n9c (OLE) and palmitoleic C16:1n7 cis-9 are prevalent, due to a large concentration in their diet and their ability of Δ^9-desaturase (stearoyl-CoA desaturase) to actively synthesize these fatty acids within the bird (Klasing, 1998). A study by Connan et al. (2005) found OLE palmitoleic acid C16:1n7 cis-9 and palmitic C16:0 (PAL) predominate in proventricular oil samples from five different species of Procellariiformes [2]. The ability of birds to synthesize fatty acids can vary relative to the level of dietary fat: some faunivorous species (animal-eating species, such as common murres) have a relatively low capacity for fatty acid synthesis by the liver compared to granivores, since their diet is rich in OLE and palmitoleic acid C16:1n7 cis-9 acid, so a high rate of synthesis is unnecessary (Herzberg & Rogerson, 1990; Klasing, 1998). Therefore, the inclusion of OLE and palmitoleic acid C16:1n7 cis-9 directly in the diet may be a more energy-efficient alternative. The requirements for fatty acids also change at different life stages. The activity of Δ^9-desaturase has been shown to increase upon sexual maturity in Japanese quail resulting in egg yolks with higher concentrations of OLE and palmitoleic acid C16:1n7 cis-9 (Pageaux et al., 1992).

Birds lack the enzymatic ability to introduce double bonds in fatty acids past the ninth carbon point (Δ^9). Only plants can introduce double bonds at the Δ^{12} and Δ^{15} points in the C18 carbon chain, making LIN and ALIN, essential fatty acids in the diet of granivorous birds. Chickens are one of the few avian species that has been widely investigated to determine their capacity to elongate and desaturate LIN and ALIN (Gregory et al., 2013). The use of vegetable oils in feeding, including soya oil, is widely practiced in the poultry industry (Saleh et al., 2021) since chickens can desaturate and elongate within hepatocytes, transforming LIN into the omega 6

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[2] The 5 species included short-tailed shearwaters *Puffinus tenuirostris*, white-chinned petrels *Procellaria aequinoctialis*, blue petrels *Halobaena caerulea*, thin-billed prions *Pachyptila belcheri*, and Antarctic prions *Pachyptila desolata*

fatty acids (ARAC) and ALIN into omega 3 fatty acids (EPA and DHA) (Gregory et al., 2013; Klasing, 1998). However, many carnivorous mammals and fish have lost the capacity for ARAC synthesis due to high availability in their natural diet, and therefore ARAC is an essential dietary requirement (Klasing, 1998). It should not be assumed that all avian species can synthesize all of the PUFA that are metabolically required and thus a prudent diet would supplement ARAC, EPA and DHA, (Klasing, 1998).

1.3 Foraging strategies

Evidence suggests that Procellariiformes regulate the duration of foraging trips during the chick-rearing as a trade-off between the cost of parental care and maintaining adult body condition (Baduini & Hyrenbach, 2003). Procellariiformes vary in the length of their foraging trips and can be described as having long, short or bimodal foraging strategies, which can involve short-distance (nearshore 1-5 day trips) or long-distance (offshore greater than 6 days) foraging trips. Bimodal foragers alternate between both long and short strategies (Baduini & Hyrenbach, 2003). The energetic cost to the parent, benefit to the chick and nutritional composition vary with the foraging strategy used.

This study investigated the diet of 3 species of burrow nesting seabirds with different foraging strategies. GFP as long-distance foragers, FS as short-distance daily feeders and CP as an endangered species that are long-distance foragers yet have a restricted range during the chick-rearing season.

1.4 Aims of the Study

a) To determine if the widely used S/Soy diet may be contributing to sub-clinical and clinical disease in translocated petrel chicks before fledging, through assessment of fledgling parameters, necropsy samples, and the success of colony establishment, when chicks are fed different artificial diets.

b) To assess what effect dietary oils have on the deposition of fatty acids in RBC phospholipid membranes in two Procellariiformes species near fledging.

c) To compare the artificial translocation diets used for burrow nesting petrels in New Zealand to the wild diet of the seabirds, using species with different foraging strategies.

d) To investigate how methods of sample collection can affect the analysis of the nutrient composition and fatty acid profiles of the diet of wild seabirds.

The chapters of this thesis are written and formatted as independent papers, so there will be some unavoidable double up in content.

Chapter Two

The effect of dietary oils on seabird chicks during translocations

2

2.1 Abstract

Translocations are a vital conservation tool used to restore natural habitats and protect endangered seabirds from environmental and human-induced impacts. In New Zealand, a diet of tinned sardines in soya oil (Brunswick™) (S/Soy) has been widely fed to *Procellariidae* species during the translocations of chicks. When this diet has been fed, there have been high fledging rates; however, when fed for longer periods, diseases have occurred related to malnutrition and may impact fledgling survival at sea.

In this study, the effect of feeding different dietary oils was compared using feeding trials that substituted soya oil with fish oil in a subset of birds during two seabird chick translocations. Fish oil supplemented diets combined with a fish analogue diet (Mazuri®) (M/Fish) were fed to 30 of 76 grey-faced petrel chicks (*Pterodroma macroptera gouldi*; kuia) (GFP) and 20 of 74 fluttering shearwaters (*Puffinus gavia*; pakahā) (FS) were fed a diet, where fish oil was added to tinned sardines in spring water (Brunswick™) (F/Fish). Over the three-week translocation period, changes in red blood cell (RBC) phospholipid membrane fatty acid ratios, fledging parameters and necropsy results were compared between dietary groups, as well as return rates thereafter. Fledging parameters were similar between the dietary groups, with S/Soy chicks fledging at significantly higher weights. Deaths due to visceral gout occurred in GFP chicks irrespective of the diet fed. Supplementing translocation diets with fish oil improved fledging RBC fatty acid ratios compared to soya oil fed chicks, reaching levels that resemble those found in the wild diet of seabirds. Fish oil supplementation achieved significantly higher proportions of docosahexaenoic acid C22:6n3 (DHA), docosapentaenoic acid C22:5n3 (DPA) and arachidonic acid C20:4n6 (ARAC), fatty acids that are important for cellular communication as eicosanoids and are vital for immune responses in birds. In contrast, soya oil fed chicks had significantly higher proportions of linoleic acid C18:2n6 (LIN), resulting in RBC membranes filled with plant-based C18 fatty acids at fledging, fatty acids that obligate piscivorous seabirds which may not be able to metabolize. S/Soy fed chicks had DHA levels that were significantly decreased, which could have a deleterious effect on chick maturation. Return rates of FS chicks to the colony showed no effect of the dietary oil-fed. Rapid changes in RBC fatty acids were observed within two to three weeks of the translocation, so it may be that nutritional differences are compensated as chicks are transitioned between wild diets. Until the exact

nutritional requirements of seabirds are known, a prudent diet would include fish oils that are already present in wild marine-based diets.

Keywords

Docosahexaenoic acid C22:6n3 (DHA), arachidonic acid C20:4n6 (ARAC), linoleic acid C18:2n6 (LIN), RBC phospholipid fatty acid, petrel, Procellariiformes, fish oil, soya oil, grey-faced petrels *Pterodroma macroptera gouldi* and fluttering shearwaters *Puffinus gavia.*

2.2 Introduction

Anthropogenic alterations on land and at sea have caused deteriorations in the conservation status of petrels and shearwaters globally, with 52 (42%) species classified as “threatened” based on IUCN criteria, and 65 (52%) species regarded as “suffering population declines” (Rodríguez et al., 2019). The translocation of petrel colonies is an effective conservation strategy used to conserve rare Procellariiformes seabirds and to restore natural habitats (Gummer, Taylor, Collen, et al., 2014). Worldwide, over 171 seabird restoration projects have been conducted in 16 countries to restore over 64 seabird species (Zhou et al., 2017), and the number of these translocations is increasing.

The success of chick translocations relies on the philopatric urge of chicks to return to their fledging nest site while attracting conspecifics to nest with them at the new colony site. Chicks are translocated to a different colony site before they have emerged, and site imprinted on their natal burrows. They are placed in artificial burrows at the new site and are gavage fed for 2-3 weeks until fledging. Acoustic signalling and decoy models are used to attract both translocated and new birds to the site. Predator control and annual supplementing through several years of chick translocations both contribute to successfully establishing new colony sites (Jones & Kress, 2012).

In New Zealand, the S/Soy diet has marked a pioneering step in the development of successful seabird translocations for burrow nesting seabirds of the family Procellariidae (petrels, diving petrels and shearwaters). The diet is both economical and practical to feed in remote locations, yet, it is not a species-specific diet. By 2009, the S/Soy diet had been used to translocate 1454 chicks from 8 different species of petrels (Miskelly et al., 2009), irrespective of their diverse natural foraging strategies and prey items consumed, from planktonic crustaceans to squid and fish. The feeding technique developed in common petrel species has been used in endangered species, including the Chatham petrel (*Pterodroma axillaris;* ranguru) and the Chatham Island petrel (Magenta petrel; *Pterodroma magenta;* tāiko) (Miskelly et al., 2009).

Fledging rates of translocated seabirds have been improving as the selection of chicks at transfer has been refined by size, shortening the time chicks are fed at the new colony site from 5 weeks to 2-3 weeks, (Gummer, Taylor, Collen, et al., 2014). Fledging weights in hand-reared chicks on the S/Soy diet have been above or close to mean natural fledging weights in seven species translocated (Miskelly et al., 2009). Determining the success of each translocation by adult return rates is logistically challenging, as colony sites are often difficult to monitor regularly, and the number of birds that perish at sea is unknown. Fledged chicks will remain at sea between 2 to 10 years before returning to either the new colony site, their source colony site, or be recruited to a different colony site with a new mate. Some colonies are less well established than others and may have lower return rates than wild colonies (pers. comm Taylor et al., 2021; Taylor, 2010).

A limitation of the S/Soy diet is that deaths potentially related to malnutrition have occurred in chicks fed this diet for prolonged periods during translocations. In translocations of GFP chicks fed the S/Soy diet, sporadic cases of hepatic lipidosis, ventriculitis, and visceral gout have all been found at post mortem (McInnes, 2007). Episodes of diarrhoea and the presence of green urates were observed in some GFP chicks before fledging (McInnes, 2007), indicative of biliverdinuria, a clinical sign of hepatic failure. These findings raise the concern that chicks with subclinical stages of nutritional disease before fledging are hard to detect and may have reduced rates of survival at sea.

The nutrient requirements of birds are only well known in domestic agricultural species (Speer, 2015) and the nutritional requirements of Procellariiformes are currently unknown. For many wild and rare species, nutrient requirements may never be determined because determining these values requires a large number of the study species, and long term and costly experiments with invasive endpoints (Speer, 2015). In the face of unknown nutrient requirements, it is prudent that artificial diets provided to wild birds reflect the natural wild diet of the species, unique features of the species' gastrointestinal anatomy, physiology, foraging and gustatory capacity, as well as; any nutritional basis for previously identified pathologies (Speer, 2015).

The oils in artificial diets fed to translocated chicks are essential for growth and performance during the long periods spent at sea (Puskic et al., 2019). A vital component of wild

Procellariiformes diets is proventricular oil, and the benefits of chicks consuming proventricular oil have been well described (Warham, 1977) (Roby et al., 1997) (Foster et al., 2010). Procellariiformes are unique among birds in storing large quantities of oil in their proventriculus that is derived from the diverse seafood items they consume, enabling the exploitation of distant food sources while incubating and raising chicks (Johnstone & Davis, 1990) (Foster et al., 2010; Wang et al., 2007) (Warham, 1977, 1996)[3].

Diet has a direct effect on the physiological functions of cells. Fatty acid proportions in RBC membranes have been widely and routinely used in mammals as biomarkers to reflect the PUFA composition of body organs and is a reliable indicator of DHA status in cardiac and brain tissue in mammals (Bradbury, 2011). Dietary oil contains fatty acids used during $_{\beta}$-oxidation for energy and in the formation of fat stores, while phospholipids form the major structural component of all cell membranes (Hussein, 2013). The fatty acid composition of phospholipid membranes can determine the magnitude of an inflammatory response when the immune system is challenged (Korver & Klasing, 1997). As the precursors of eicosanoids, very long-chain (PUFAs) improve cell membrane fluidity and cell communication (Harrison & Lightfoot, 2006; Klasing, 1998). Dietary intake of the omega-6 PUFA (ARAC) leads to the production of pro-inflammatory signalling molecules, including prostaglandins, thromboxanes and leukotrienes, while omega 3 PUFA (DHA, EPA and DPA) have a potent anti-inflammatory effect preventing many chronic disease processes, including atherosclerosis and osteoarthritis (Heinze et al., 2012; Hussein, 2013; Klasing, 1998; Speer, 2015). Early exposure during chick growth to DHA and ARAC is crucial for the optimal functioning of cells, immunoregulation and development of brain, retina, skeletal and cardiac tissues (Cherian, 2015) (Koppenol et al., 2014). In poultry, adding fish oil to the diet has been shown to have positive effects on growth and lessened the growth-suppressing impact of disease exposure by improving the immune responses (Korver & Klasing, 1997).

This study compares the effect of artificial diets on two species of Procellariiformes with distinctly different foraging strategies. GFP are long-range foragers, where parent birds may forage at sea for several days (>5 days) as parents exploit distant foraging habitats (Baduini & Hyrenbach, 2003). In contrast, FS are short-range foragers who take shorter foraging trips and feed their chicks daily. GFP are cephalopod specialists that also consume fish and crustaceans in appreciable quantities (Imber, 1973) and forage live prey by using surface seizing “sit and wait” tactics and “dipping” while also opportunistically scavenging large dead or moribund

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[3] All Procellariiformes produce proventricular oil apart from diving petrels *Pelecanoididae.*

prey (Imber, 1973). GFP are primarily nocturnal foragers, enabling them to exploit vertically migrating bioluminescent prey. In contrast, nocturnal feeding is less important for *Puffinus* species like the FS (Imber, 1975). FS forage over the continental shelf and inshore waters, mainly by pursuit-plunging and predominantly taking fish as prey (Gummer & Cotter, 2012). Juveniles disperse to waters off the eastern and south-eastern Australian coasts, while adults are largely sedentary in New Zealand coastal waters year-round (Gummer & Cotter, 2012).

The frequency and volume of chick feeding during translocation are modelled on the species translocated foraging strategy; FS chicks are fed daily and GFP chicks are fed every other day. The feeding frequency of parent birds is known to decline as fledging approaches, so translocation feeding regimes aim to mimic the growth curve of wild colony chicks by increasing chick weight to above adult weight and then decreasing it by the time chicks fledge (Gummer, Taylor, et al., 2014a; Gummer, Taylor, Collen, et al., 2014).

This thesis hypothesised that birds fed the S/Soy diet will have RBC phospholipid PUFA ratios that are significantly different to the ratios found in wild piscivorous diets; and, that birds fed the fish oil supplemented diet will have similar RBC phospholipid PUFA ratios as the wild phenotype.

2.2.1 Aims

1. To determine if the widely used S/Soy diet fed to chicks may be contributing to sub-clinical and clinical disease in translocated chicks before fledging through the assessment of fledgling parameters, necropsy samples, and the success of colony establishment when chicks are fed different artificial diets.

2. To assess what effect dietary oils have on the deposition of fatty acids in RBC membranes in two Procellariiformes species near fledging.

2.3 Materials and methods

2.3.1 Feeding trials

Grey-faced petrel translocation

During a Cape Sanctuary and New Zealand Department of Conservation (DOC) translocation, 76 GFP chicks were translocated 200km from Moutohorā Island (37°51'27.0"S 176°58'17.7"E) in the Bay of Plenty, to The Cape Sanctuary (39°41'03.5"S 177°03'32.9"E) in the Hawkes Bay, on the 3rd December 2010 (Figure1). The transfer protocol followed DOC guidelines regarding feeding volumes, frequency, and daily monitoring (Gummer, Taylor, Collen, et al., 2014). The size range of chicks selected for transfer aimed to select chicks that have not emerged from their burrows to avoid imprinting on their natal colony site, while also being as close to fledging age as possible to minimise the amount of time chicks are fed at the new colony site. Specific chick selection criteria included 200-240mm wing lengths and a minimum weight of 500g. Further details of burrow design, acoustic signalling, and monitoring of pre-fledging emergence are detailed in Miskelly et al. (2009).

Figure 1. Map of New Zealand showing petrel colony sampling sites.

The dotted arrow shows the 200km translocation distance for grey-faced petrels (GFP) from the wild source colony at Moutohorā Island (Δ) to the new translocation colony site (dark Δ) at Cape Sanctuary. (◊) shows the GFP Wild colony control site at Te Henga (Bethells Beach). The solid arrow shows the 50km translocation distance for fluttering shearwaters from the (○) wild source colony at Long Island to the translocation colony site (●) at Matiu/Somes Island.

On arrival, a physical examination was undertaken on all translocated birds and general assessments immediately before every feed to identify any obvious health concerns. Throughout the study period, morphometric data were collected with wing lengths measured by metal rulers from the carpometacarpus to the primary feather tip, and body weight measured using calibrated spring scales to weigh the birds in fabric bags. Feather condition, food consumption, and behaviour during handling were routinely recorded, along with any abnormal findings, including unusual behavioural responses, regurgitation, and faecal consistency in the burrow. The date chicks emerged from their burrows, and fledging dates were recorded. Necropsies and histology were performed on any deaths that occurred over the translocation to fledging period.

In the previous two seasons of GFP transfers, randomised disease screening for *Salmonella*, *Yersinia* and *Campylobacter* species and faecal egg counts for parasites all returned negative, so disease screening was not undertaken in this season.

Because this research was undertaken opportunistically during a conservation translocation, some components of the dietary management of the birds were dictated by established protocols and therefore birds were not placed into the feeding trial groups until day 3 of the translocation, further details of the initial management of the birds are provided below. context.

All chicks were gavage fed using clear plastic tubing (Figure 2), given 20ml of Lactated Ringers solution for hydration on arrival at the translocation site, and blocked into individual artificial burrows. On the second day following translocation, all chicks were fed 50ml of the S/Soy diet (Table 1,

Table 2), diluted with an additional 50ml of water added to the recipe for this first feed. From day three, chicks were split into two groups using stratified, random sampling based on wing length for the feeding trial. The two groups of chicks were fed either S/Soy or a powdered fish analogue diet designed by Mazuri® specifically for piscivorous birds that eat high-fat fish (M/Fish), with additional fish oil added (Table 1).

Watties Fish Oil™ (Bakels Edible Oils, New Zealand) was added to the M/Fish diet from day 5 and fed throughout the study (Table 1, Table 2). Due to delivery delays, an alternate fish oil was added to the M/Fish diet on days 3 and 4, (Healtheries Fish oil low odour 1500mg Vitaco Health New Zealand, Auckland, New Zealand). Special care was taken to ensure the fish oil was properly stored, including the addition of 0.5mg dl-α –tocopherol (DSM, Netherlands) to prevent rancidity. In the GFP trial, oil was split into 250g samples that were subsequently sealed in foil laminate pouches in a temperature-controlled room. The pouches were placed into a C200 Multivac (Multivac, Germany) and a vacuum pulled down to 50 mbar. The pouches were then flushed with nitrogen until a pressure of 400 mbar, sealed and returned to atmospheric pressure. In the FS trial, to ensure the fish oil was not exposed to light or oxidation and for ease of transport, the oil was stored in a keg (Cornelius ball lock keg - 19 L) that had been industrially cleaned before use. Carbon dioxide was used to pressurise the keg to prevent exposure to oxidation and light and the keg was kept in a cool storage place.

The Mazuri fish analogue® portion of the M/Fish diet is a powdered diet formulated for fish-eating birds and was made up on-site with boiled water and subsequently cooled, as per manufacturer's instructions. The S/Soy diet was prepared daily in electric blenders off-site and transported to the colony site in a refrigerated bin within 3 hours. Both diets were cooled or warmed up respectively to be fed at body temperature (38-40°C). The volume of fish oil added was calculated to equal the proportion of fat found in the S/Soy diet, which was calculated by draining the tins and measuring the volume of oil per tin. The Mazuri diet was selected as an alternative diet after comparing it with the results of a previous GFP nutritional analysis study (Hendriks et al., 2000) using the Zootrition nutritional computer programme.

Figure 2. Gavage feeding of a grey-faced petrel chick

The schedule of the diets used are presented in Table 2. As detailed above, both diet groups were fed the S/Soy diet on arrival at the translocation site. To minimise complications associated with transitioning diets, the M/Fish diet group was slowly transitioned onto the M/Fish diet over 3 days, increasing increments of 33% a day and reaching 100% M/Fish diet by the fifth day. On the sixth day, the feeding interval was extended to every other day, replicating the natural 'long foraging' feeding strategy of GFP, as is standard GFP translocation protocol. (Gummer, Taylor, Collen, et al., 2014).

Table 1. The two artificial diets used in the grey-faced petrel feeding trial

Ingredients	**DIET**	
	S/Soy	**M/Fish**
Fish	106g Canadian sardines in 11% soya oil (Brunswick™), juvenile Antarctic herrings *Clupea harengus.*	25g Mazuri high fat, fish-eating bird, gel Diet ® 50/27 analogue diet™ ("Mazuri")
Boiled water	50ml	75ml
Additives	1/3 x 0.19g seabird supplementary vitamin (Mazuri ® Vita-Zu vitamin)	6ml fish oil*

Table 2. The feeding schedule of grey-faced petrel chicks fed two different artificial diets at the beginning of the translocation

Day	**Diet S/Soy (GFP, n=46)** **Sardines in Soya oil**	**Diet M/Fish (GFP, n=30)** **Mazuri in Fish oil**
1	20ml isotonic saline	20ml isotonic saline
2	50ml diluted diet S/Soy*	50ml diluted diet S/Soy*
3	50ml S/Soy	33ml S/Soy + 16.5ml M/Fish
4	100ml S/Soy	33ml S/Soy + 66ml M/Fish
5	100ml S/Soy	100ml M/Fish
6 +	50-100ml S/Soy, EOD (●)	50-100ml M/Fish EOD (●)

GFP = grey-faced petrels; EOD = every other day

(●) First blood samples taken after transfer at 6-10 days.

** To ensure adequate hydration, the first feed was modified at a ratio of 100ml water per 106g tin sardines instead of 50ml water*

Feeding volumes varied based on each chick's morphometrics (feathering/growth rate) and behavioural response to the feed. In preparation for fledging the chicks were more likely to regurgitate during a feed so lower volumes were fed near estimated fledging dates. Mazuri Vita Zu® vitamins are routinely added to the sardine diets S/Soy diet. Each 0.19g Mazuri Vita-Zu® tablet contained vitamin A (835IU), vitamin E (25IU), vitamin C (25mg), thiamine (24mg) riboflavin (1.8mg), pyridoxine (1.8mg), pantothenic acid (1.8mg,) folic acid 60mcg,bBiotin 30mcg, no less than 1% crude protein, no less than 0.5% crude fat and no more than 0.5% crude fibre.

Fluttering shearwater feeding trial

A second feeding trial was run during a translocation of 80 FS chicks that were moved 50 km from Long Island, Marlborough Sounds (41°06'40.1"S 174°17'35.3"E) to Matiu/Somes Island (41°15'38.1"S 174°51'53.3"E), Wellington Harbour, on 8th January 2012 (Figure 1). Chicks selected had wing lengths between 150-183mm and weights ranging from 270-505g.

Health assessments and ongoing monitoring were undertaken as outlined for the GFP trial. Routine screening of all blood-sampled chicks (n=30) was negative for *Plasmodium* spp. (avian malaria) and *Erysipelothrix* spp. by PCR and cloacal swabs were taken for microbiology screening for *Salmonella* spp. and *Campylobacter* spp. Faecal egg count of chicks were found to be negative for nematodes, with 3 chicks showing a low burden of coccidia (from 28 random birds sampled with 13 repeat samples).

A total of 74 FS were placed in the feeding trial. The first 20 chicks in burrows numbered 1-30 were allocated to the S/Fish group, and the rest were placed in the S/Soy group. The S/Soy dietary group contained (n=54) chicks that were fed the S/Soy, as previously described Table 1. While (n=20) birds were fed a modified Brunswick tinned sardine in spring water™ diet, drained, with fish oil added (S/Fish), (Table 3). Fish oil was stored as previously described.

Table 3. The alternative diet (S/Fish) used during the fluttering shearwater feeding trial.

Ingredients	**DIET S/Fish**
Fish	106g Canadian sardines in 10% spring water and salt (Brunswick™) drained, juvenile Antarctic herrings *Clupea harengus.*
Boiled water	50ml
Oil added	** Watties™ Fish Oil, Bakels Edible Oils, New Zealand, 15-20ml.
Additives	1/3 x 0.19g seabird supplementary vitamin (Mazuri ® Vita-Zu vitamin)[1]

*** The oil was calculated as equal to that in the S/Soy diet (17ml per 106g tin). As the trial progressed the volume of fish oil added increased to 20ml for the first 14 days and reduced to 15ml until fledged, to reduce the oily consistency of the faeces.*

1 Mazuri Vita Zu® vitamins are the same as the S/Soy diet.

Table 4. Fluttering shearwater dietary groups and transiting feeding schedule of the first 4 days on the artificial diets.

Day	Diet S/Soy (FS, n=54) Sardines in Soya oil	Diet (S/Fish) (FS, n=20) Sardines in Fish oil
1	25ml isotonic saline	25ml isotonic saline
2	30ml diluted diet S/Soy*	30ml diluted diet (S/Fish)*
3	30-80ml S/Soy (●)	30-80ml (S/Fish) (●)
4+	30-80ml S/Soy daily	30-80ml (S/Fish) daily

FS = fluttering shearwater, EOD = Every other day

(●) First blood samples taken after transfer at 3-4 days.

**In order to ensure adequate hydration, the first feed was modified at a ratio of 100ml water per 106g tin sardines instead of 50ml water for GFP and diluted with 75ml per tin instead of 50ml in the FS diet trial.*

Feeding volumes vary based on each chick's morphometrics (feathering/growth rate) and behavioural response to the feed. In preparation for fledging the chicks are more likely to regurgitate during a feed so lower volumes are fed near estimated fledging dates.

The FS were fed isotonic saline on arrival and then given a diluted first feed om the second day (Table 4). However, unlike in the GFP feeding trial, the FS chicks were fed once daily and diets with different oils were fed from the first feed, with no soya oil transition in the diet.

Feeding volumes of 30-80ml were adjusted relative to bird metrics (using wing length as a guide), aiming for a weight gain of 5-10g a day and fledging weight of >385g (Gummer, 2012). Volumes were reduced near fledging, to mimic wild colony weight loss and to minimise the risk of regurgitation that occurs near fledging, because it can damage the waterproofing of mature feathers. Volumes were also reduced if the chick approached 500g, as previous translocations have shown that heavier weights can delay fledging, (Gummer, Taylor, Collen, et al., 2014). Methods of food preparation, meal size, meal delivery, hygiene measures, burrows and emergence follow DOC protocols (Gummer, 2012; Gummer & Cotter, 2012; Gummer, Taylor, et al., 2014b).

2.3.2 Red blood cell phospholipid membrane fatty acid analysis

GFP blood sampling and handling

To assess the phospholipid composition of RBC membranes, paired blood samples (n=10 from S/Soy; n=7 from M/Fish) were taken from a random selection of translocated GFP chicks at 6-10 days after transfer ("mid-transfer") and again 14-18 days later immediately pre-fledge, ("pre-fledge"). A random selection of 44 chicks was sampled at mid-transfer and 26 at pre-fledge, however, due to early fledging and sample damage during cryopreservation, only n=17 chicks with paired blood samples were included in this study.

Samples were taken from conscious birds at the medial metatarsal or ulna wing vein using a 25g needle and 1ml or 3ml syringe, with volumes taken less than 1% of body weight (ranging from 0.6ml to 2.5ml). Samples were transferred to a 0.6ml lithium heparin collection tube (BD Microtainer®, BD Vacutainer Systems, NJ, USA). The packed cell volume (PCV) and total protein (TP) were assessed on the day of collection, using a mini centrifuge (Labnet Internation Inc, Prism min centrifuge 600rpm), and the remaining sample was transferred into a cryotube, centrifuged, and the plasma removed. The RBC were then washed twice in 1ml of isotonic saline by gently agitating, centrifuging, and removing the saline with a pipette. The RBC pellet was then transferred to a cryotube and 1.7 ml of butylated hydroxyl toluene (BHT) in methanol reagent was added, at a concentration of 5mg BHT/100ml methanol. The sample was agitated with a pipette until the contents turned a homogeneous light brown colour. Blood samples were transported in liquid nitrogen and stored at -80°C.

In addition, blood samples were taken, as previously described, from a separate wild colony of chicks (n=11) (Wild) on Ihumoana Island and neighbouring Kauwahaia Island (36°53'27.7"S 174°26'22.0"E), in the Waitakere ranges 26-28 November 2010 (Figure 1). The site was chosen as a control colony because the colony's fledging parameters and return rates had been closely monitored in the preceding 20 years (pers. comm Taylor, 2010). Blood samples were taken from chicks with wing lengths ranging from 222 to 279mm and weights ranging from 540-940g to enable comparison to the GFP trial population.

FS blood sampling and handling

To assess RBC phospholipid fatty acid composition, paired blood samples were collected (S/Soy, n=9; S/Fish, n = 10), as previously described, although smaller volumes of blood were used (0.3-1.0ml). Samples were taken at 3-4 days after arrival ("mid-transfer") (

Table 2) and again near predicted fledging dates 13-14 days later ("pre fledge"). Measurements of weight and wing length of blood-sampled chicks were compared on arrival and 20 days later, except for one chick that fledged at 19 days.

Laboratory analysis of RBC phospholipid fatty acids

Massey University Nutrition Laboratory, Palmerston North, New Zealand conducted RBC fatty acid analysis using methods adapted by Michelle McGrath and Adrienne Portch referencing (Williams, 2006) and (Harris, William 6-6-08.doc).

In brief, red blood cell pellets of various volumes that were stored in liquid nitrogen were placed in individual screw cap silica glass tubes. For each 50µl of red blood cells, 1000µL of 3N methanolic HCL and 200µL of internal standard (IS) working solution was added. The IS stock solution consisted of 22.5mg +/-0.5mg of heptadecanoic acid-dissolved in 100ml methanol and was further diluted to 22.5µg/ml by adding 2500µl to 25ml of methanol to form the IS working solution.

Samples were incubated for four hours at 90˚C with occasional mixing and then left to cool to room temperature before transfer into 10 or 15ml kimax tubes suitable for the centrifuge. A further 2000uL of hexane was added to each sample of 50µl of red blood cells sample; samples were shaken manually for 10 minutes, vortexed for 1 minute and then centrifuged at 2000 rpm and 20˚C for 3 minutes. The top layer was removed with a disposable Pasteur pipette and transferred to another clean screw cap silica glass tube with a Teflon seal. Samples were dried in a Buchi Syncore™ Polyvap parallel evaporator because these samples have a low hexane volume. Samples were dissolved by adding 100ul per 50ul red blood cells of hexane per 50ul red blood cells to each tube, using a calibrated pipette.

Samples were then transferred to gas chromatography vials, using Pasteur pipettes. Fatty acid analysis was run using a gas chromatograph, (model Shimadzu G17a). Wash bottles in the gas chromatograph autosampler were filled with hexane and 5 uL of the sample was injected into a gas chromatograph. The concentration of fatty acids in the sample was calculated by comparing the average area of the sample to the concentration and area of the internal standard in the standard solution.

$$\begin{aligned} &mg\ fatty\ acid\ per\ ml\ of\ sample \\ &\qquad = (\bar{x}\,area\ of\ spl\ \times\ \bar{x}\ area\ of\ IS\ in\ Std\ Sol \\ &\qquad \times conc\ of\ Std\ sol\ \times DF\ of\ spl\ \times conc\ IS\ Sol) \\ &\qquad \div (\bar{x}\ area\ of\ Std\ \times \bar{x}\,area\ IS\ in\ Sample \\ &\qquad \times conc\ of\ IS\ Std) \end{aligned}$$

KEY
$\bar{x}$= average
Spl= sample
IS= internal standard
Std Sol=Standard Solution
Conc= Concentration

Dietary oil fatty acid analysis

The oils of each dietary group were analysed at the Massey University Nutrition Laboratory, Palmerston North, New Zealand. Fatty acid analysis was run using a FAMEs, gas chromatograph separation, based on Sukhija & Palmquist, 1988, (model Shimadzu GC-17A). By this analysis, the limit of detection of fatty acids was 0.01g/100g and the limit of quantification 0.03g/100g.

The M/Fish diet was formulated in the GFP trial after comparing analysis of GFP regurgitation samples published by Hendriks et al. (2000) with several alternative fish-eating bird diets, using the software programme (Zootrition Software, St. Louis, MO, USA). The Mazuri High fat (frozen gel) 50/27® was selected as a possible diet substitute for the S/Soy. Additional fish oil was added to equal the volume of oil in the S/Soy diet, after comparisons with a previous study by Hendriks et al. (2000) showed there was substantially higher fat in the wild diet.

2.3.3 Statistical analysis

Assessment of fledging data.

Using Minitab, Anderson Darling tests were used to check for normality. Wing length, body weight and days before fledging were compared between dietary groups of each species, GFP S/Soy (n=40) and M/Fish (n=26) and between FS S/Soy (n=51) and FS S/Fish (n=19), using paired t-tests or Mann-Whitney tests for non-parametric samples.

RBC phospholipid fatty acid analysis

GFP and FS blood samples were analysed to quantify 36 fatty acids, comparing only samples from chicks that had a paired ‘mid-transfer’ and ‘pre-fledge’ sample. The data set was converted into a percentage of total fatty acids. Fatty acid values that were less than the value of quantification (LOQ= 0.001mg/ml) were analysed by assigning values of 0.00001mg/ml which is below the limit of detection (0.0002mg/ml).

In both the GFP and FS blood samples myristic acid C14:0 could not be well separated from the BHT peak during analysis, so the exact volume of the fatty acid is unknown. Since this is a saturated fatty acid, it has little effect on PUFA comparisons. Siince the myristic peak is very far from the DHA peak, it should not affect any comparisons of this particular fatty acid. The data was analysed both including and excluding myristic acid without significantly different results, therefore myristic acid was removed from the data set entirely and the total percentage of fatty acids were calculated without including it, to ensure a more accurate representation.

Individual fatty acids were checked for normality with probability plots and Anderson darling tests. Data that were not normally distributed and could not be transformed to become so, using log transformations (Log10, antilog, natural log, exponential), arsine, square root or box cox transformations. Therefore, permutational multivariate analysis of variance (PERMANOVA) and non-parametric analysis was used.

PERMANOVA analysis of fatty acids

All RBC fatty acid variables were similar and used the same scale, so no transformation or standardisations were deemed necessary. Principal component analysis (PCA) was run and the variables (fatty acid percentages) were overlaid as vectors. Only variables with a very high degree of correlation with the PCA, axes were plotted ($r > 0.85$).

Hypothesis tests were then performed in PERMANOVA (Anderson, 2001) (McArdle & Anderson, 2001) to explore the effects of two fixed factors (*Diet, Time*) and one random factor (*Bird*) on the blood fatty-acid profiles of the seabirds. In GFP The PERMANOVA analysis was initially run including each chick (Bird) as a random effect to allow for individual-level variation in fatty acid profiles between the individual birds but the term was firmly non-significant ($p > 0.5$) and the estimated variance component was zero, so the term was dropped from the model.

PVA analysis of wing lengths and weights

Wing lengths and weights used in the PCA comparisons were taken from the day before blood sampling in the GFP group and on the day of sampling in the Wild control group. The FS wing length and weight comparisons were taken on the day of arrival and compared to 20 days later, except one chick that fledged at 19 days.

Non-parametric analysis of significant RBC fatty acids.

Fatty acids of particular interest to nutrition and fatty acids with significant difference were compared using non-parametric analysis using the R statistical computer program. The difference in means was tested by using the simple 95% bootstrap confidence intervals between diet groups and changes within individuals, using library desk tools in (R, 2021).

The difference in the level of fatty acids in diets and RBC membrane fatty acids were analysed, specifically looking at nine key fatty acids of interest. Fatty acids included: C22:6n3 – cis-4,7,10,13,16,19 Docosahexaenoic acid (DHA), C22:5n3 – cis-7,10,13,16,19 Docosapentaenoic acid- (DPA), C20:5n3 – cis-5,8,11,14,17- Eicosapentaenoic acid (EPA), C24:1n9 – cis-15- Nervonic acid (NERV)**,** C20:4n6 – cis-5,8,11,14-Arachidonic acid (ARAC)**,** C18:2n6c Linoleic acid (LIN), C18:3n3 – cis-9,12,15-Alpha linolenic (ALIN), C18:1n9c Oleic acid (OLE) and C6:0 Caproic acid (CAP).

2.3.4 Post-mortems

All trial birds that died during the translocation period were refrigerated and transferred to Massey University, Palmerston North, for post mortem examination by a veterinary pathologist with additional histological examination of tissues from all chicks and adjunctive microbiology, as indicated.

2.3.5 Return rate assessment

The Matiu/Somes FS colony was checked monthly since 2012, with band checks of any returning adults and subsequent DNA feather sexing undertaken. This screening aimed to detect any of the 237 FS chicks that were translocated over the 2012-14 period from Long Island (pers. comm Cotter, 2021).

GFP return rates have been monitored by the number of birds returning in total and by banding new chicks. Adult bands are being opportunistically monitored, however the catching of adults has generally been avoided in order to encourage adults to recolonise undisturbed.

2.4 Results

2.4.1 GFP feeding trial

Eighty-six percent (66/76) of birds included in this study were retained in the final sample population (S/Soy dietary group, n=40; M/Fish dietary group, n=26). Chicks were removed due to deaths from injury (n=2), predation (n=2), illness (n=3), fledged early in a storm (n=1), and disappearance from the burrow at an age that the chick was unlikely to have successfully fledged (n=2). *(For more detail see 2.4.15 post mortem findings).*

2.4.2 GFP growth parameters: wing length, weight and days before fledging.

Transfer morphometrics

At the beginning of the trial period, there was no evidence of a difference between the two treatment groups (S/Soy and M/Fish) in terms of mean wing lengths (t=1.06, p=0.292) or mean transfer length (t=0.35, p=0.72). At transfer, the wing length and weight parameters were normally distributed in the 66 GFP chicks that fledged.

Fledging morphometrics.

Comparison of the birds in the different dietary groups at fledging showed no evidence that the diet fed was affecting the number of days spent on-site (Mann Whitney w=1302.5, p= 0.624) or wing length at fledging (Mann Whitney w= 1418, p=0.308). At fledging, chicks fed the S/Soy diet were significantly heavier than chicks fed the M/Fish diet (p=0.002) (Figure 3, Figure 4 and Table 5).

The majority of the fledgling morphometrics were within the target aims previously reported by Gummer et al, (2014) (Table 5). There were some chicks in both diet groups that fledged with wing lengths below the minimum target of 305 mm but were still within the range of wild colony fledging wing-lengths.

Table 5. Fledging parameters of grey-faced petrel chicks fed different rearing diets from translocation to fledging and the statistical tests used to compare groups.

Fledging parameters	GFP Diet group S/Soy n=40	GFP Diet group M/Fish n=26	Target transfer & fledging aims *	A Wild Colony **	Transfer Diet Comparison					
					P value ▪	CI	Difference	W value ▪	T value	df
Transfer weight (g)					*Two sample t-test*					
Mean +/- SD	654 +/- 104	626 +/- 104	>500g		*0.292*	*(-24.7, 80.50*	*27.9*	*NA*	*1.06*	*53*
Median	647.5	600								
Range	(450-930)	(500-950)								
Mean error	17	20								
Transfer wing length (mm)					*Two sample t-test*					
Mean +/- SD	220.9 +/- 10.6	219.8 +/- 15	200-240 mm		0.727	*(-5.18, 7.39)*	*1.1*	*NA*	*0.35*	*64*
Median	220	220								
Range	(195-242)	(190-248)								
Mean error	1.7	2.9								
Fledging weight (g) on day before fledge					*Two sample t-test*					
Mean +/- SD	586 +/- 46.3	554.6 +/- 33.3		510 +/-22.9	*0.002*	*(11.79, 50.98)*	*31.38*	*NA*	*3.2*	*63*
Median	577.5	550								
Range	(505-730)	(500-630)	(520-550) a (500-600) b	(395-700)						
Mean error	7.3	6.5								
Fledging wing length (mm) on the day before fledge					*Mann Whitney*					
Mean +/- SD	306.9 +/- 9.51	303.3 +/- 12.4		294 +/- 4.18	*0.308*	*(-2,7)*	*3*	*1418*	*NA*	*NA*
Median	307.5	307								
Range	(278-320)	(273-316)	(315-320) a (305-340) b	(266-323)						
Days spent on site.					*Mann Whitney*					
Mean +/- SD	20.1 +/- 3.72	20.64 +/- 4.77	≥ 23		*0.624*	*(-2, 2)*	*0*	*1302 .5*	*NA*	*NA*
Median	20	22								
Range	(12-27)	(12-29)	(15-34)							

*▪ Adjusted for ties. Outliers have been removed. Weights represent pre-feed weights. Transfer dates include the day of transfer. *(Gummer, Taylor, Collen, et al., 2014). (a) Represents the average range of weights and wing lengths of parent reared chicks from the Te Henga, West Auckland, (the location of our wild control colony), this is the preferred target range for translocated chicks. (b) is the generally accepted range for all GFP translocations. The preferred weight range at fledging is slightly heavier than the usual weights for GFP adults (480-580g) at Te Henga, however, observations for Moutohorā Is GFP where the translocated chicks were sourced from are heavier (470-605g) (Gummer, Taylor, Collen, et al., 2014; Imber, 1976). ** Wild colony parameters represent all chicks (n =15) that fledged in the same season from the Te Henga control colony, including potentially unsuccessful fledges, courtesy of (Dunn, 2012), these results were considered below average results compared to previous seasons.*

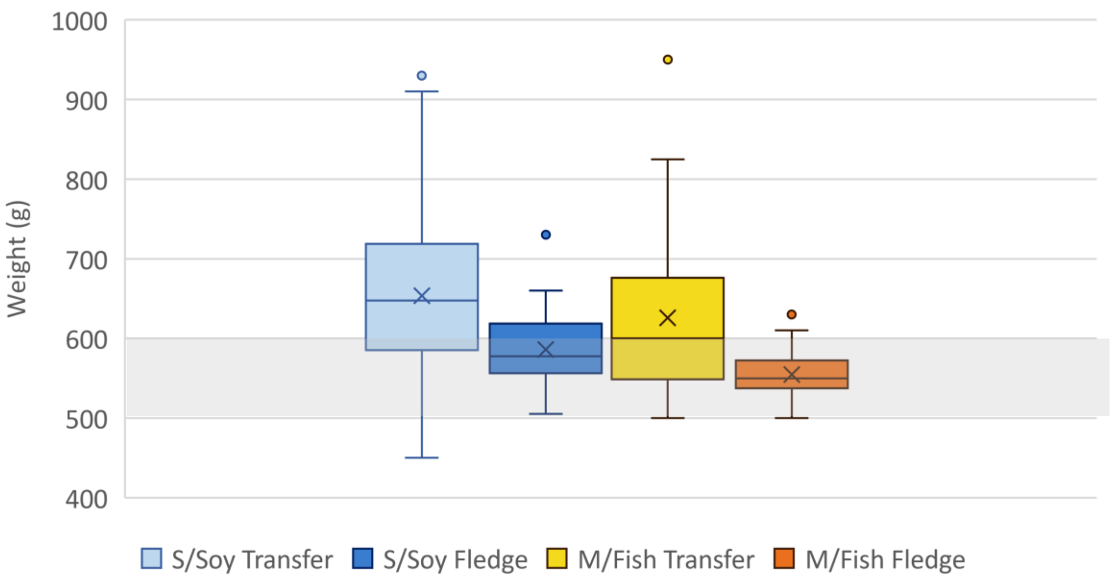


Figure 3. Boxplot showing the difference in grey-faced petrel transfer and fledging weights of healthy chicks that fledged on both diets.

Chicks fed the (S/Soy) diet were significantly heavier at fledging (p=0.002) than(M/Fish) fed chicks. Weights are expected to decrease near fledging. The grey shaded area on the graph represents the preferred weight range for chicks at fledging. (Gummer, Taylor, Collen, et al., 2014)

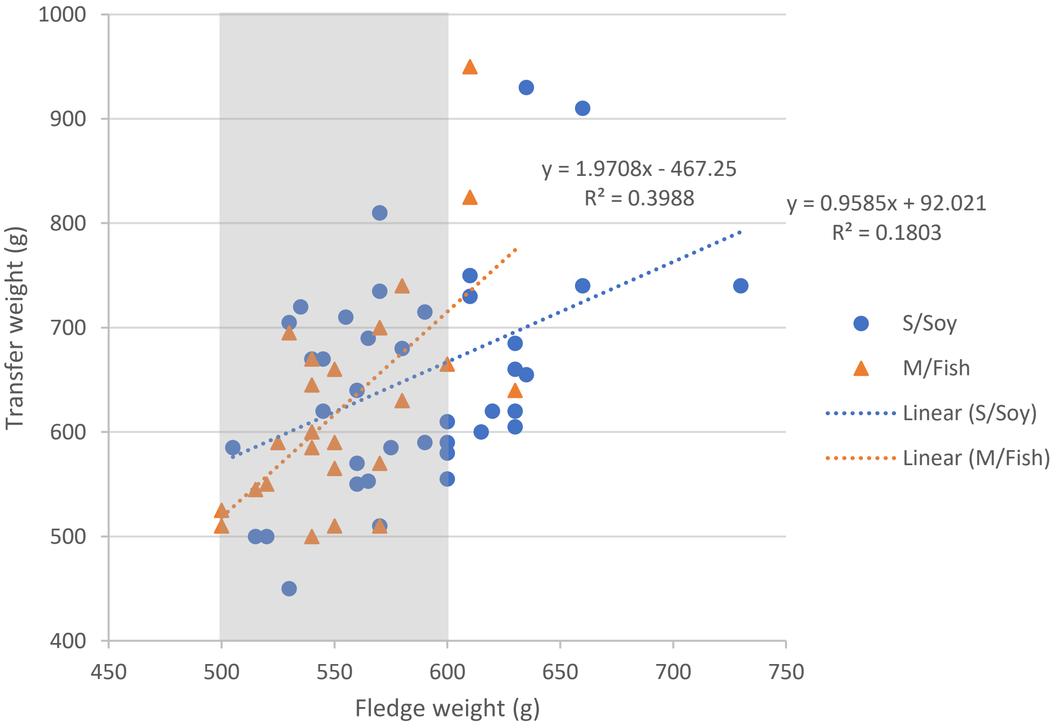


Figure 4 Healthy grey-faced petrel chick weights at transfer and fledging, while being fed either the S/Soy (n=40) or M/Fish (n=26) diet.

The grey shaded area represents the preferred weight range for chicks at fledging.

(Gummer, Taylor, Collen, et al., 2014)

2.4.3 GFP analysis of RBC fatty acids

In total, 17 paired blood samples (mid-transfer and pre-fledge) were available for RBC fatty acid analysis, n=10 S/Soy fed chicks and n=7 M/Fish fed chicks. In addition, 11 single blood samples were analysed from Wild fed chicks. Overall, 35 fatty acids were successfully identified in the samples. Both time and diet had strong effects on the ratios of fatty acids in chicks. PCA plots depicted the dietary groupings well, with 73% of variation explained by the first principal component (Figure 5) (Table 6). Wild-fed GFP chicks had higher proportions of OLE, ARAC, and DHA, and lower proportions of LIN, than chicks fed artificial diets, whereas pre-fledge S/Soy chicks had higher proportions of LA and lower proportions of OLE, ARAC and DHA. When first transferred the RBC fatty acid proportions of chicks fed the M/Fish and the S/Soy diets were more similar to each other than to the chicks fed the Wild diet.

Mid-transfer samples

Despite the initial similarity of artificial diet groups with the Wild diet, there was a clear divergence between RBC fatty acid groups (Figure 5). Mid-transfer samples were quite similar to the Wild dietary group and reflected the difference in diets over the first 6 - 10 days of the transfer (Table 2). Mid-transfer chicks on the M/Fish diet that had been fed artificial diets for 6 days had RBC fatty acid ratios that were more similar to Wild diets, where proportions of LIN and ALIN in this diet were lower (Figure 5, Figure 6). There is a clear shift evident in the mid-transfer samples: as the percentage of soya oil in the diet increases, so does the average LIN percentage (2.74% WILD diet < 8% M/Fish < 12.8 % S/Soy diet) (Figure 6).

Pre-fledge samples

By the time of the pre-fledge samples, the pattern of fatty acids in RBC of GFP fed the S/Soy diet were notably different to the M/Fish fed birds, which remained more similar to the Wild fed chicks (Figure 5, Figure 6). The fatty acids most accountable for the similarities between Wild fed and M/Fish-fed chicks were OA, ARAC and DHA, whereas the proportion of LA in chicks fed the S/Soy diet was primarily responsible for the difference with other groups. The S/Soy-fed chicks had high LIN and lower OLE, ARAC, DHA compared with mid-transfer S/Soy chicks and M/Fish-fed chicks at both mid-transfer and pre-fledge (Figure 5, Figure 6).

Notable outliers

At mid-transfer there are two notable outliers in the S/Soy group. In figure 6, the PCA plot is annotated to identify chicks S3 and S4, both of which started closer to and trended rapidly towards the same ratios as the S/Soy pre-fledge group over this time (Figure 5). The chicks S3 and S4 had the lowest body weights within the S/Soy early transfer group, while their wing lengths were in the middle and lower quartile of the group at transfer.

At the pre-fledging time point, there was another prominent outlier in the S/Soy group (S10, Figure 5), whose RBC fatty acids sat centrally within the M/Fish dietary group, with RBC fatty acid ratios more similar to the M/Fish and Wild samples (showing higher DHA, ARAC, OLE and lower LIN). S10 was notable as the heaviest chick selected from the source colony in the S/Soy group, weighing 910g (100g heavier than any other chick and 400g more than the lightest chick in the group) (Figure 7). The wing length of S10 was not remarkable (220mm) at selection for transfer out of the wild colony.

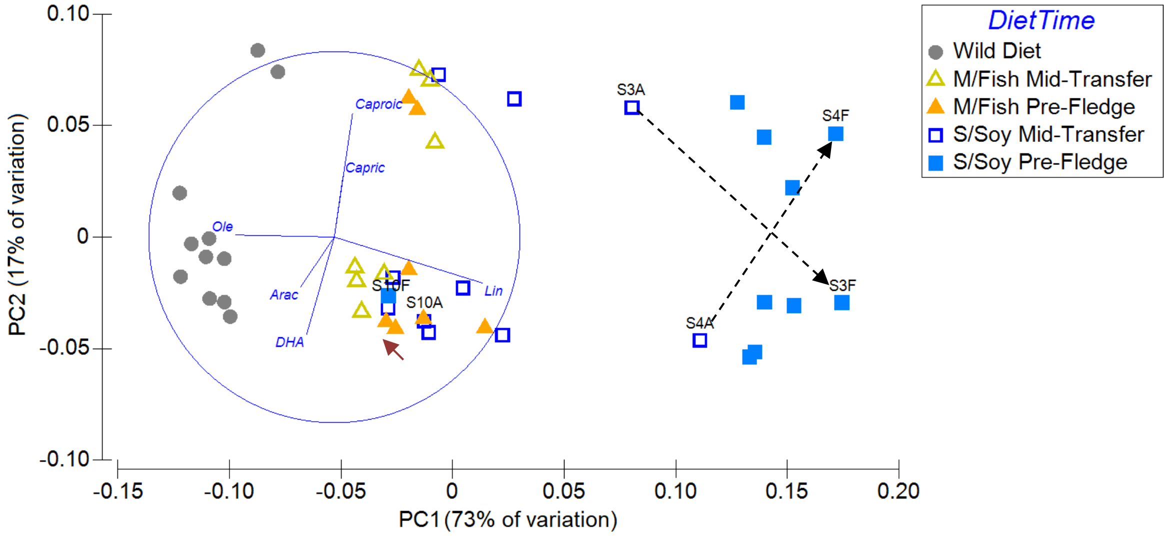
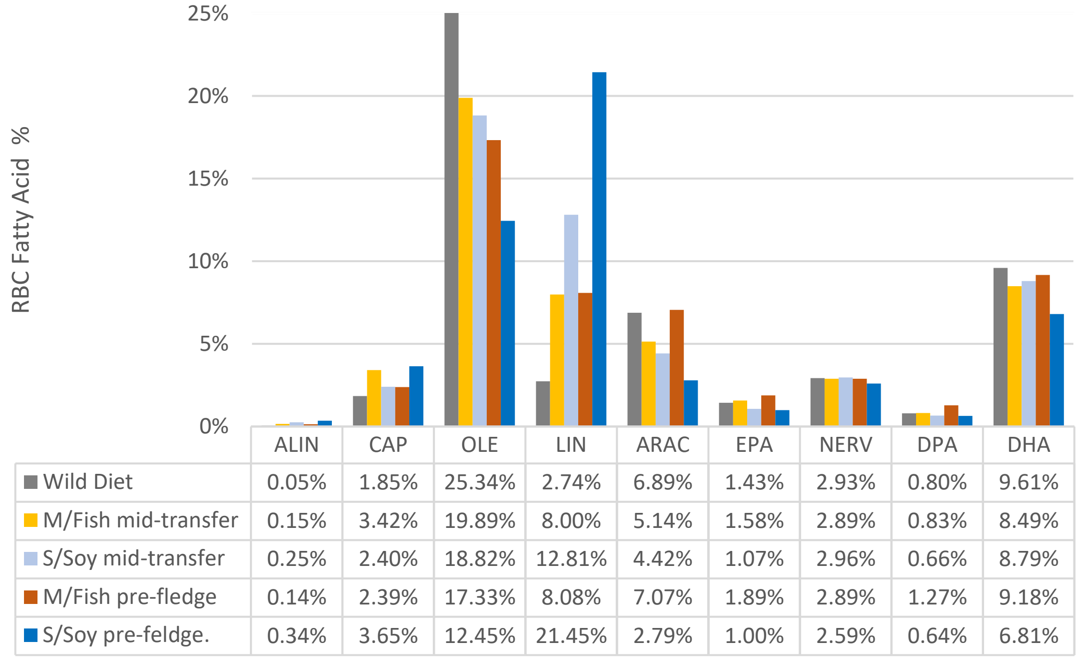


Figure 5 PCA plot of grey-faced petrel RBC phospholipid fatty acids ratios in chicks fed either the Wild diet at one point in time or the M/Fish and S/Soy over 14-18 days (between mid-transfer and pre-fledge samples).

Outliers S3, S4 and S10 are labelled with arrows to show the transition from (A) mid-transfer arrival samples and (F) pre-fledge samples

The black dashed arrows show the direction of fatty acid changes over the transfer period for S3 and S4, who were both fed the S/Soy diet for 4 days longer before blood sampling, compared to all other chick's blood sampled at 6 days. S10 is a S/Soy fed chick with a pre-fledge sample that travels in the opposite direction to all other S/Soy chicks sampled (red arrow) with fatty acid ratios that are more similar to the M/Fish and WILD fed chicks.

The PCA plot captures the vast majority (90%) of the total fatty acid variation seen in GFP, (73% on the x axis and 17% on the y axis), therefore we can be confident that the differences we see in the plot accurately reflect the true variation in the bird's fatty acid profiles.



	ALIN	CAP	OLE	LIN	ARAC	EPA	NERV	DPA	DHA
■ Wild Diet	0.05%	1.85%	25.34%	2.74%	6.89%	1.43%	2.93%	0.80%	9.61%
■ M/Fish mid-transfer	0.15%	3.42%	19.89%	8.00%	5.14%	1.58%	2.89%	0.83%	8.49%
■ S/Soy mid-transfer	0.25%	2.40%	18.82%	12.81%	4.42%	1.07%	2.96%	0.66%	8.79%
■ M/Fish pre-fledge	0.14%	2.39%	17.33%	8.08%	7.07%	1.89%	2.89%	1.27%	9.18%
■ S/Soy pre-feldge.	0.34%	3.65%	12.45%	21.45%	2.79%	1.00%	2.59%	0.64%	6.81%

Figure 6 Changes in grey-faced petrel mean RBC phospholipid fatty acid proportions of key fatty acids of interest between dietary groups over the transfer period.

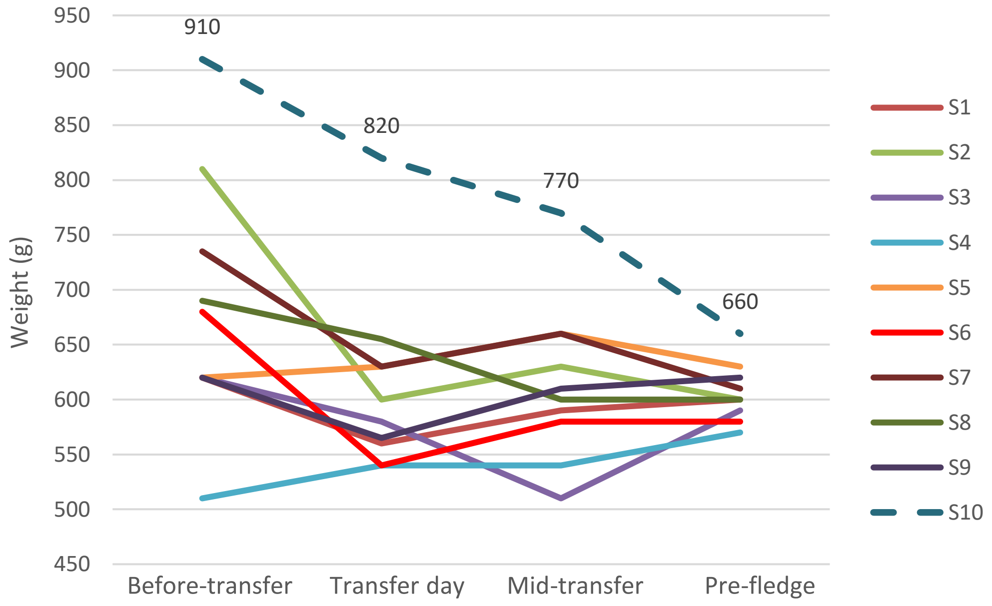


Figure 7 Difference in weights of S/Soy fed chicks, comparing transfer weight to fledging, with outlier S10 labelled as this was the heaviest, most recently parent-fed chick in the group.

Figure 7 shows the decline in weight over a transfer period as the chicks prepare to fledge, S10 was the heaviest chick at selection and through-out the transfer period. Weighing 910g at selection and 820g two days later at transfer, (165g heavier than the other chicks in the group). WL from transfer to fledge grew from 229 to 315mm and was not the longest WL at any point. At each blood sample S10 was still the heaviest chick in the group but by fledging the difference in weight had diminished to only 30g heavier than the next largest chick.

2.4.4 GFP PERMANOVA of RBC fatty acid profiles

The RBC fatty acid profiles were significantly different between chicks fed the two artificial diets fed in this study, and the difference changed between mid-transfer and pre-fledge samples (Table 6; Pseudo-$F_{1,30}$ = 5.3, p = 0.0052).

Table 6 PERMANOVA table of grey-faced petrel red blood cell phospholipid fatty acid profiles comparing chicks over time and diet. Analysing Wild (n=11) control colony samples at a single point in time and paired blood samples of dietary groups S/Soy (n=10), M/Fish (n=7), at mid-transfer and pre-fledging.

Source	Df	SS	MS	Pseudo-F	P(perm)	Unique perms
Diet	1	0.0815	0.0815	18.386	0.0001	9944
Time	1	0.0407	0.0407	9.1829	0.0001	9952
Diet x time	1	0.0235	0.0235	5.2973	0.0052	9936
Res	30	0.133	0.0044			
Total	33	0.289				

Table 7 Estimates of components of variation for grey-faced petrel RBC phospholipid fatty acid analysis.

Source	Estimate	Sq.root
S(Diet)	0.0046793	0.068405
S(Time)	0.0022023	0.046929
S(Diet x Time)	0.0023131	0.048095
V(Residual)	0.0044328	0.066579

There were differences in RBC fatty acid profiles between diet groups: even when accounting for the effect of time, the effect of diet was large compared to the residual variation between individual birds. *Diet* played the largest role in determining the fatty acid profile of the birds' RBC, followed by residual variation, and the interaction term between variables (Table 7).

2.4.5 GFP individual RBC fatty acid comparisons at different stages of the transfer

As fledging approached, changes in the mean fatty acid proportions in chicks fed artificial diets became more distinct from the Wild dietary group, the greatest changes were observed in the fledging diet of S/Soy chicks, while other key fatty acids showed very little change at all (ALIN, CAP, NERV and EPA), (Figure 6, Figure 8 - Figure 13).

In summary, ALIN, CAP and NERV did not significantly change between any of the diet groups or between multiple samples from individual chicks. ALIN had very little variation while CAP showed a wide variation with individuals of the same diets and between diets (Figures 8 - 16).

OLE was the predominant fatty acid in the Wild dietary group (Figure 6), with significantly more OLE than artificial diets at all stages of the transfer, occupying 25% of RBC membranes (Figure 6, Figure 8-12). Average OLE proportions were not different between artificial diets at mid-transfer, but then significantly decreased over time in both diets, especially in chicks fed the S/Soy diet where the mean percentage had halved by the pre-fledge sample (12.5%) (Figure 6, Figure 8-12).

LIN was significantly higher in both the mid-transfer groups (M/Fish 8.1% and S/Soy 12.8%) compared to the relatively low level in the Wild dietary group (2.7%), (Figure 6, Figure 8- Figure 13). The elevation of LIN is consistent with the higher proportion of LIN in the oils of both artificial diets fed by 6-10 days into transfer (Table 2). Overtime in the M/Fish dietary group, the proportion of LIN stayed constant at 8% while the S/Soy group had a substantial increase in LIN reaching an average of 21.45% near fledging (Figure 6, Figure 12, Figure 13).

ARAC was significantly higher in Wild diets compared to mid-transfer artificial diets, (Figure 6, Figure 8, Figure 9). There was no difference in artificial diets at mid-transfer, however, as feeding progressed ARAC proportions in the M/Fish chicks significantly increased to match levels found in the Wild diet, (mean% Wild 6.89% = M/Fish 7.07%) while S/Soy chicks decreased to significantly lower levels (2.79%) (Figure 6, Figure 8-13).

EPA in wild diets was comparable to both artificial diets at all stages of the transfer yet, the amount of EPA in the S/Soy fledge sample showed a decreasing trend while the amount of EPA in the M/Fish fledged samples showed an increasing trend over time. The artificial diets were not significantly different at mid-transfer, but there was a strong trend towards higher EPA in the M/Fish group. The M/Fish chicks had a significantly increased EPA percentage by pre-fledging compared to S/Soy-fed chicks (Figure 6, Figure 8-13). There was very little variation in EPA between individuals on each diet (Figure 14-16).

DPA proportions stayed relatively constant between diets until pre-fledging when DPA in chicks fed the M/Fish diet significantly increased, to levels above both the S/Soy diet and the Wild diet (mean % 1.27% M/Fish pre-fledge > 0.8% wild diet ≥ 0.64% S/Soy pre-fledge) (Figure 6, Figure 8-14). Between diets, the individual variation was not significant but trended towards S/Soy chicks having lower DPA (Figure 14-16).

DHA was highest in the Wild diet (9.61%) at all stages of the transfer. There were no significant differences between levels of DHA among artificial diets at 6-10 days into the transfer (mean % =8.49% M/Fish and 8.79% S/Soy). Over the course of the transfer levels of the M/Fish diet increased slightly, staying similar to the Wild diet (9.18%), while S/Soy fed chicks had a significant decrease (to 6.81%) compared to both the Wild and M/Fish diets (Figure 6, Figure 8-13). Individual variation was not significant although there was quite a wide variation between individual chick DHA (Figure 14-16).

Wild diet comparisons at mid-transfer

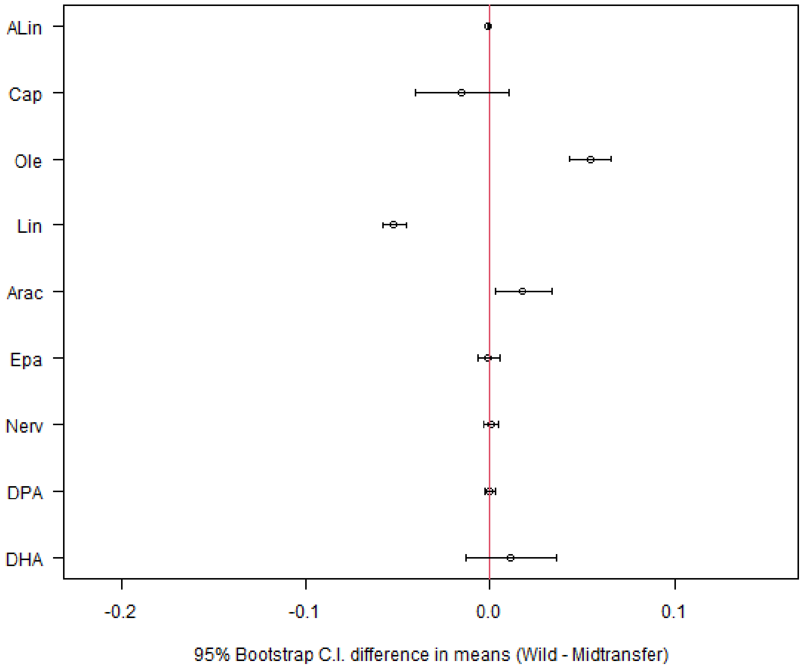


Figure 8 Wild compared to M/Fish dietary groups at mid- transfer. *

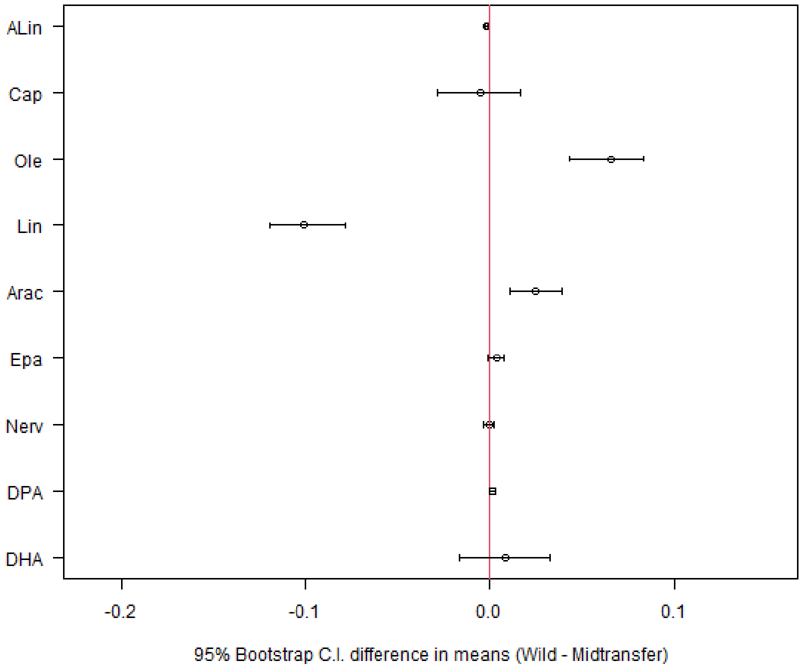


Figure 9 Wild compared to S/Soy dietary groups at mid-transfer. *

**Changes in mean % of RBC phospholipid fatty acids in grey-faced petrel chicks, graphed by bootstrap confidence intervals. Error bar represents the 95% confidence interval.*

Wild diet comparisons at pre-fledge

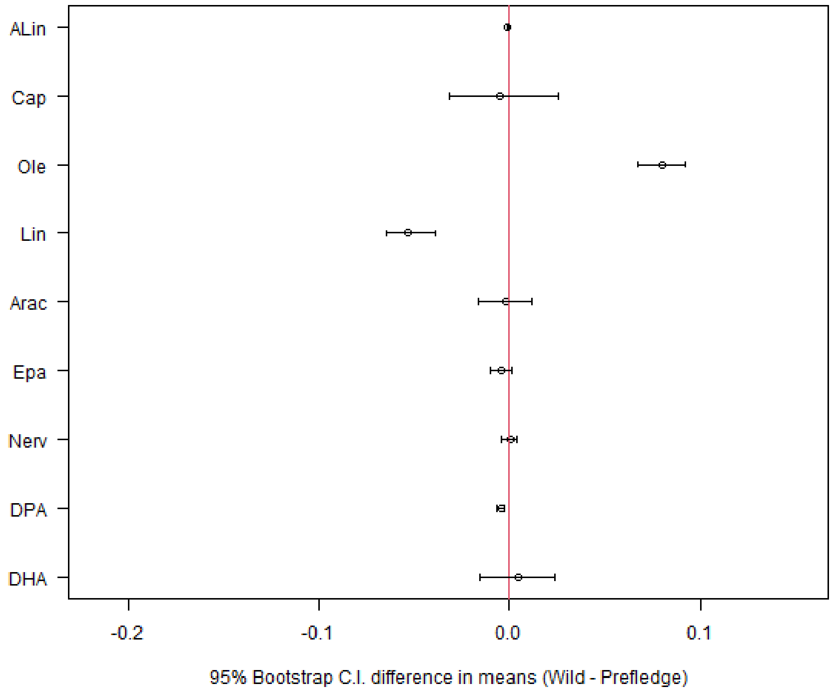


Figure 10 Wild compared to M/Fish dietary groups at pre-fledging. *

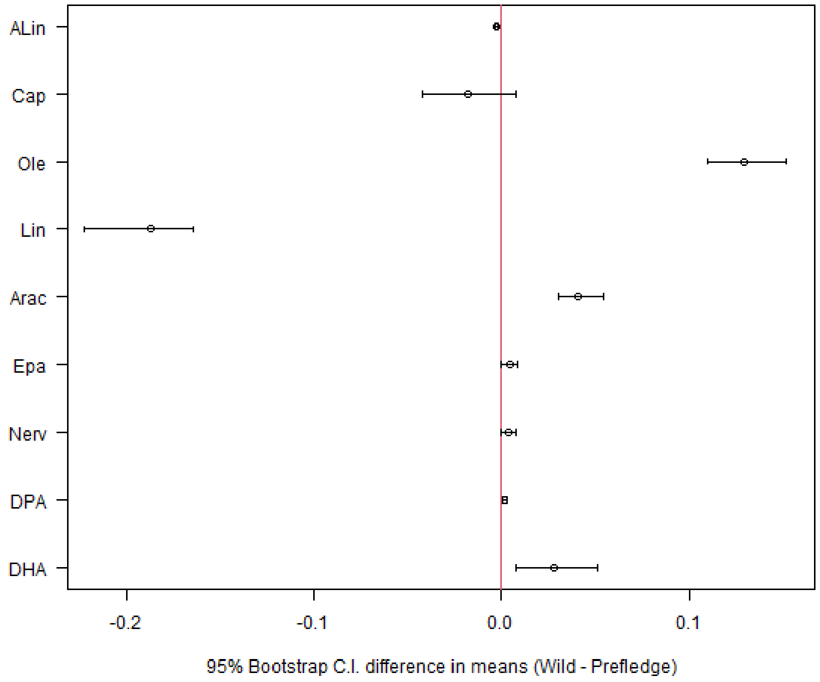


Figure 11 Wild compared to S/Soy dietary groups at pre-fledging. *

**Changes in mean % of RBC phospholipid fatty acids in grey-faced petrel chicks, graphed by bootstrap confidence intervals. Error bar represents the 95% confidence interval.*

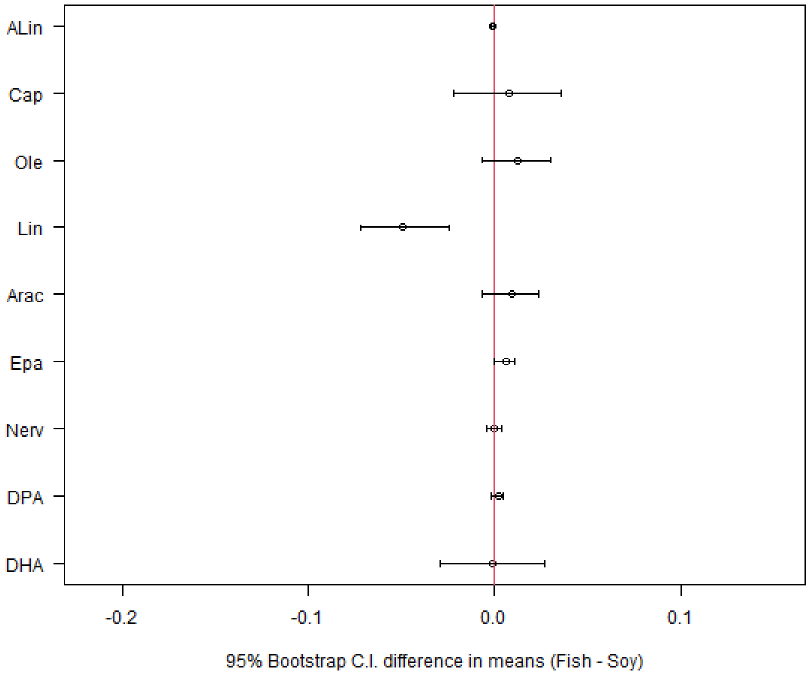


Figure 12 Difference between dietary groups M/Fish and S/Soy at mid transfer. *

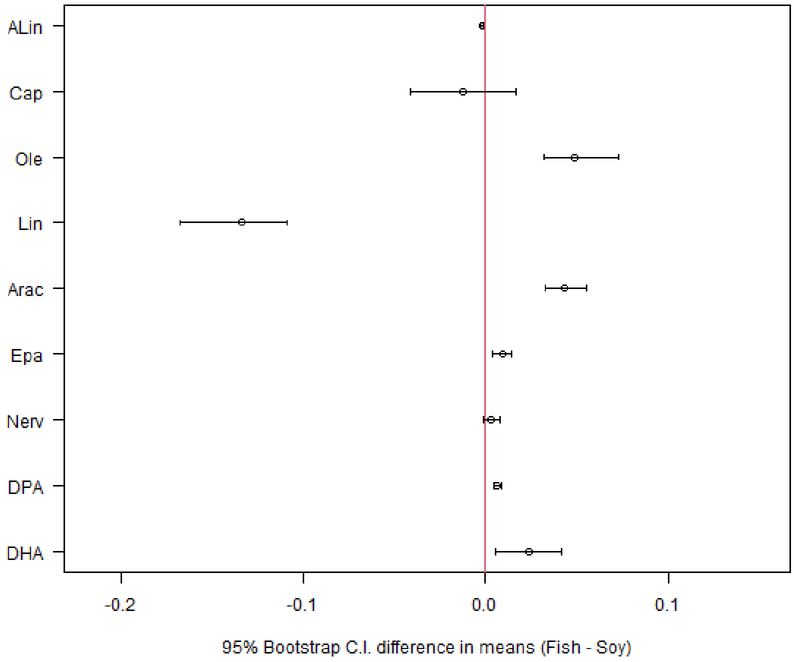


Figure 13 Difference between dietary groups M/Fish and S/Soy at pre-fledge. *

**Changes in mean % of RBC phospholipid fatty acids in grey-faced petrel chicks, graphed by bootstrap confidence intervals. Error bar represents the 95% confidence interval.*

Changes within individual chicks

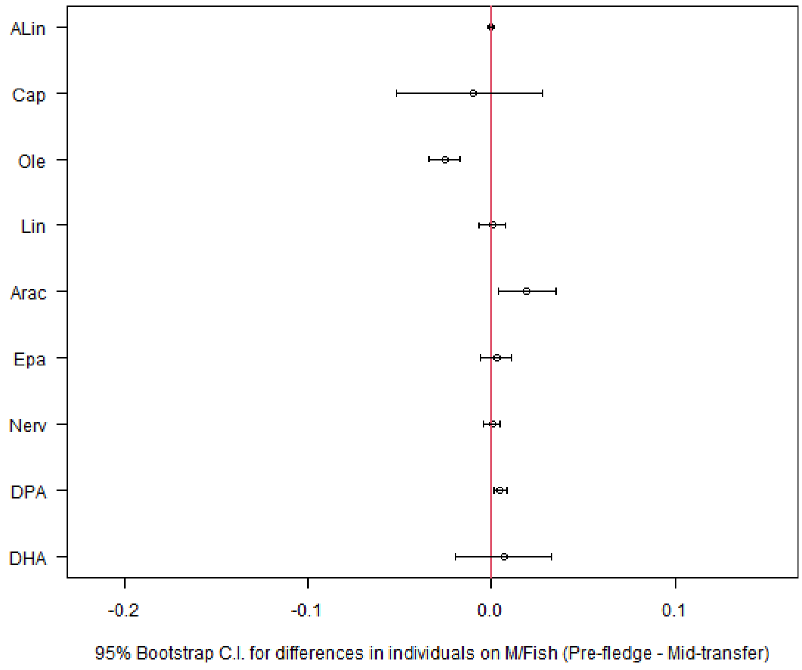


Figure 14 The effect of feeding the M/Fish diet on individual chicks over the translocation from mid-transfer to pre-fledge. *

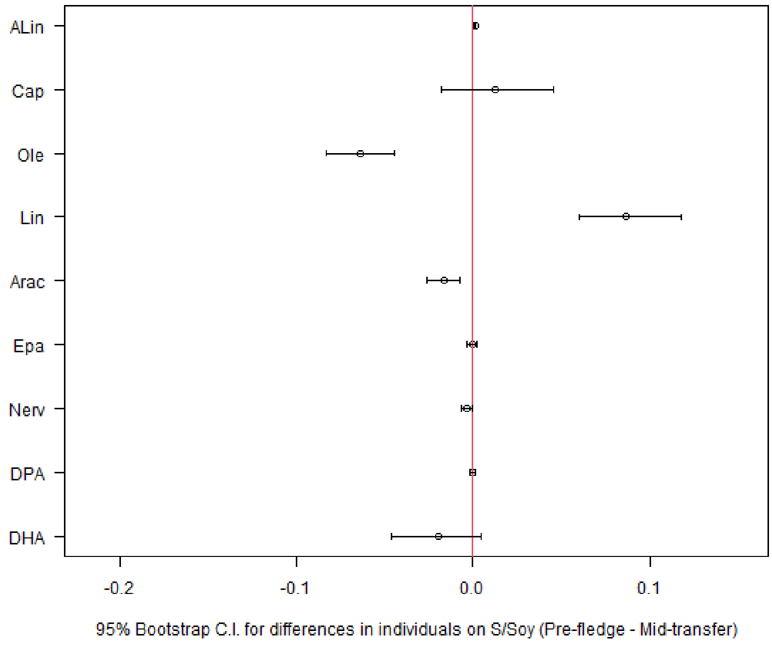


Figure 15 The effect of feeding the S/Soy diet on individual chicks over the translocation from mid-transfer to pre-fledge*

**Changes in mean % of RBC phospholipid fatty acids in grey-faced petrel chicks, graphed by bootstrap confidence intervals. Error bar represents the 95% confidence interval.*

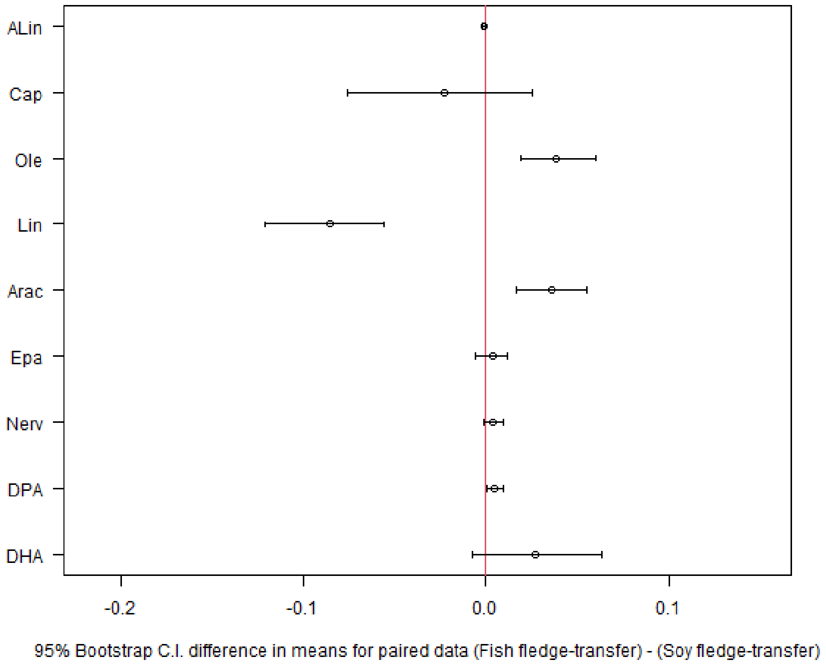


Figure 16 Differences in individual chicks, between dietary groups M/Fish and S/Soy, over the transfer period*

**Changes in mean % of RBC phospholipid fatty acids in grey-faced petrel chicks, graphed by bootstrap confidence intervals. Error bar represents the 95% confidence interval.*

2.4.6 GFP Return rates

The GFP colony has been slower to establish than expected and monitoring of adults has not been undertaken to avoid deterring nest establishment of recolonising adults. As a result recruitment rate of the feeding trial chicks as adults is unknown. The colony has been expanding, in the 2020/21 season, over 30 chicks were banded. As of May 2021, 63 burrows were being prospected from nesting this season.

2.4.7 FS feeding trial

The FS translocation recorded 92.5% (74/80) of birds as successfully fledged from the colony. Six chicks died from heat stress during the transfer. Of 74 chicks considered to have fledged in the study, 96.2% (52/54) (n=52) were retained in the S/Soy dietary group and 100% (20/20) (n=20) in the S/Fish dietary group.

FS health assessment

Five chicks had abnormal health parameters during the transfer, but all fledged successfully (Table 8). Two of the chicks were excluded from the dietary trail as they were sent off-site for treatment at a wildlife clinic and fed a different diet before returning to fledge from artificial burrows. One chick had a mild inflammatory response near fledging and was treated prophylactically on-site. Two chicks had minor health concerns that did not require treatment (Table 8).

Probable fledges

Statistics comparing successful fledging and return rates can be strongly affected by the criteria used to count a ‘successful fledge’. DOC protocols recommend identifying the birds considered to be ‘probable fledges’. These chicks that disappeared early with one or more possible complications, including down feathers still present, low weights, short wing lengths or minor health concerns, are still presumed to have fledged at a later date from vegetation around the colony. Ten chicks were reported as probable fledges (Table 8) (Gummer, 2012). At the time of transfer the target weights considered necessary for chicks to successfully fledge was thought to be above 385g, however, several chicks that were below weight have since successfully returned to the colony.

Table 8 The criteria for inclusion or exclusion of fluttering shearwater chicks in the statistical analysis groups. Translocation variables that may affect fledging and return rates are described, noting any chicks that had health concerns, below average fledging criteria, missing data or are known to have perished.

Fluttering Shearwater Outliers						Statistical Analysis Groups Selection					
Group	**n=**	**Diet**	**# Blood sample / Burrow**	**Reason**	**Returned to colony**	**Fledging parameters**	**Fledging rate (%) ^**	**Return rate to colony (%)**	**PCA**	**PERMANOVA**	**Individual FA analysis**
Died	6	NA	NA	Heat stress during transfer	NA	X ☠			NA		
Illness	3	S/Soy + SAM	13	Bill injury. Ear infection. Medicated off site▪	N	X◊					
			S1/50	Corneal scratch + respiratory signs. Medicated on site. Feather waterproofing treatment off site after pre-fledge blood sample.	N	X◊			✓~	X	X
		S/Soy	S4/ 62	Lethergy and mild lymphocytosis noted at pre-fledge blood sample. Medicated and improved rapidly. Fledging from burrow was delayed for treatment.	N	X◊	✓	✓	✓~	✓	✓
Missing data	1	S/Fish	F7/12	Pre-fledging wing length taken 4 days prior.	N	X	✓	✓	✓	✓ (WL excluded)	✓
Minor health concern	2	S/Fish	F8/14	Sneezing on arrival, NSF, WBCC WNL.	N	✓					
		S/Soy	58*	Markedly lipaemic mid-transfer blood sample-serum nearly white. -NSF, WBCC WNL	N	✓			NA		
Fledged and perished	3	S/Soy	28	Fledged, then found in Wellington with head injury 4 days later.	N	✓☠					
			78	4 years after fledging found dead on Oriental bay beach, Wellington. Never seen at colony.	N	✓☠					
			88*	23 days after fledge was found dead on 90 mile beach, Loch Sport, Victoria, Australia. Transferred at below recommended wt.	N	✓☠					
Probable fledged from vegetation	10	S/Fish	6	<10% down	N	✓					
			19	<10% & 15% down cover at fledge.	N						
		S/Soy	52	< 15% down cover	N						
			57	<70% down cover- but last down record is 5 days old	N						
			68	<50% down cover	YES						
			70	<20% down cover	N						
			76	<25% down cover	YES						
			86	<25% down cover	N						
			58*	Lipaemia blood sample. WBCC WNL	N						
			88*	<10% down, found under flax day before disappearing.	N						
Regurgitated in transfer box.	1	S/Soy	92	NSF. All other chicks that regurgitated in the transfer boxes died of head stress.	YES						
Fledged below target weight	3	S/Soy	77	77, 88 were small chicks with wing lengths at the lower end of the known fledging range, weights were 372g and ≤ 375g.	N						
			88*		N						
		S/Fish	27*	Average wing length with low weight 373g.	YES						
Coccidia positive	3	S/Fish	20	Positive to for low burden of coccidia. None treated.	Yes						
			27*		Yes						
		S/Soy	65		Yes						

✓= included or X= excluded from analysis

SAM= hospital diet of pureed salmon slurry with vitamins added.

^ Fledging rate only includes chicks that remained on-site to fledge from their burrows and were not fed the hospital diet SAM at any time.

◊ - blocked into burrow near fledging for treatment or observation therefore fledging parameters may be artificially prolonged.

~ Included as a discussion point in the PCA graphs- exclusion did not affect PERMANOVA statistics significantly

** Chick in more than one outlier group*

NA= not applicable

NSF = No other significant findings on general physical exam by a veterinarian and fledging parameters were normal.

WBCC WNL= White blood cell count within normal limits.

☠ =Confirmed dead.

▪ This chick was found wandering on the surface at the wild colony- it would have been site imprinted and in retrospect, it should not have been transferred.

2.4.8 FS return rates

For this study, calculations of fledging rates relative to return rates only include chicks retained within the nutritional study groups, excluding any chicks that had complications unrelated to diet (i.e. those that died during transfer (n=6) or were fed a different diet (n=2)) (Table 8). Therefore, the fledging rate on both diets was 100% (S/Soy n = 52; S/Fish n = 20).

By April 2021, the return rate of chicks from this cohort that returned to the colony in the 9 years since transfer includes 18 birds fed the S/Soy diet 34.6% (18/52) and 8 birds fed the S/Fish diet 40% (8/20). At the time of writing, there is no significant evidence (X^2=0.18, P = 0.67) of a difference in the return rates between the dietary groups S/Soy and S/Fish.

2.4.9 FS growth parameters

87.5% (70/80) of birds were retained in the fledging parameters group after chicks were removed due to (n=6) death, (n=3) illness or treatment that delayed fledging and (n=1) missing data. The number of birds retained for analysis of fledging parameters was 51 in the S/Soy group and 19 in the S/Fish group.

Wing length and weight at transfer and fledge were not significantly different between diets. There was variation in weight groups with the S/Fish group weighing in slightly but not significantly lighter (mean = 375, s.e. +/- 13) than the S/Soy group at transfer (mean = 402, s.e. +/- 5.9), (p=0.075), (Table 9, Figure 17, Figure 18). The number of days the chicks remained on-site before fledging was not significantly different: S/Fish (mean = 24, s.e. +/-1) and S/Soy (mean = 22, s.e. +/- 0.75), (Table 9).

Table 9 Fledging parameters of fluttering shearwaters from transfer to fledge with the statistical analysis used to compare dietary groups S/Soy and S/Fish.

Fledging parameters	S/Soy n=51	S/Fish n=19	Target transfer and fledging aims	Transfer Diet Comparison					
				P value *(adj for ties)*	CI	Difference	W value *(adj for ties)*	T value	df
Transfer weight (g)				*Two sample t-test*					
Mean +/- SD	402+/-42	375 +/-55	○	*0.075*	*(-2.8,54.7)*	*25.9*	*NA*	*1.86*	*26*
Median	365	370							
Range	183-500	270-505							
Mean error	5.9	13							
Transfer wing length (mm)				*Two sample t-test*					
Mean +/- SD	168+/-9	165+/-8	○	*0.307*	*(-2.10,6.49)*	*2.19*	*NA*	*1.04*	*36*
Median	167	167							
Range	152-183	152-175							
Mean error	1.2	1.7							
Fledging weight (g)				*Two sample t-test*					
Mean +/- SD	433+/-24	429+/-24		*0.465*	*(-8.38-,17.93)*	*4.77*	*NA*	*0.74*	*32*
Median	439	434							
Range	372-480	373-471	>385*						
Mean error									
Fledging wing length (mm)				*Mann Whitney*					
Mean +/- SD	214+/-6	216 +/-5		*0.16*	*(-5,1)*	*-2*	*1704*	*NA*	*NA*
Median	215	216							
Range	200-223	204-226							
mean error	0.8	1.1							
Days spent on site.				*Mann Whitney*					
Mean +/- SD	22+/-5	24+/-4		*0.068*	*(-5, -0.00)*	*-2*	*1672.5*	*NA*	*NA*
Median	23	25							
Range	11-31	13-31	>18days						
Mean error	0.75	1							

○ Target selection criteria on transfer day. Wing lengths determines the minimum transfer weights. Wing length of 150-160mm = weight must be >300g, 161-170 mm WL = >340g and 171-180mm WL= >380g. (Gummer & Adams 2010

*The target fledging weight of >385g is from (Gummer, 2012), however, * chicks have since returned to this colony with fledging weights of as low as 373g.*

Fledging wing lengths were taken within 24-48 hours of fledging from burrows, while weights were taken the day before fledging.

Outliers removed include n=6 dead chicks, n=2 chicks medicated off-site (#13 & S1/#50), n=1 chick blocked in to finish treatment (S4/#62) and n=1 chick with wing length data > 4 days old (F7/#12).

Figure 17 Fluttering shearwater weight changes from transfer to fledge, comparing the S/Soy and S/Fish diet.

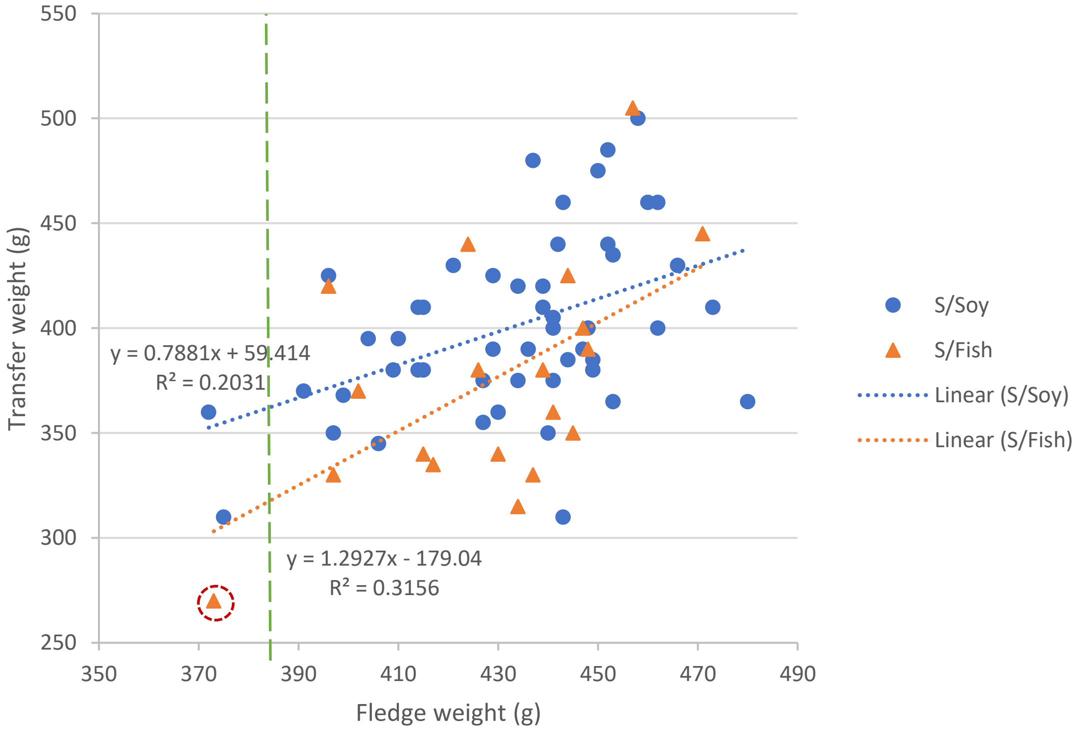


Figure 18 Fluttering Shearwaters change in weight from transfer to fledge, comparing the dietary group's sardine in soya oil S/Soy with the sardines in fish oil S/Fish.

The green dashed line (---) represents the aim for fledging weights (>385) in 2012 (Gummer, 2012), while the red dashed circle (O) is a chick that has since returned to the new colony.

2.4.10 FS RBC fatty acid analysis

In total, 19 paired blood samples were taken from FS, including n=9 chicks fed the S/Soy diet and n= 10 chicks fed the S/Fish diet. Two chicks in the S/Soy group showed signs of illness. S1 was kept in the PCA plot as an interesting outlier but was excluded from all statistical analyses (Table 8, Figure 19, Figure 20). S4 was noted as being occasionally lethargic at day ten after the transfer, whilst still being vocal and eating well. At the pre-fledge blood sample, a mild inflammatory response (mild lymphocytosis) was noted on a blood smear but, all other findings, including biochemistry and the veterinary clinical exam, were within normal limits. As a precaution, the chick was treated for five days with enrofloxacin (10mg/kg PO bid, Enrotril™ 25mg/ml, Ethical Agents Manukau, New Zealand) for general malaise of unknown cause. The chick recovered rapidly and fledged. During the blood sampling period, no treatment was given so this chick has been included in the PCA and PERMANOVA analysis (Table 8, Figure 19 Figure 20, Table 10, Table 11).

One chick from the M/Fish dietary group (F7) was excluded due to missing data. The chick has been included in the PERMANOVA analysis and with the wing length excluded, (Table 8, Figure 19, Figure 20, Table 10 Table 11).

2.4.11 FS PCA analysis of RBC fatty acids.

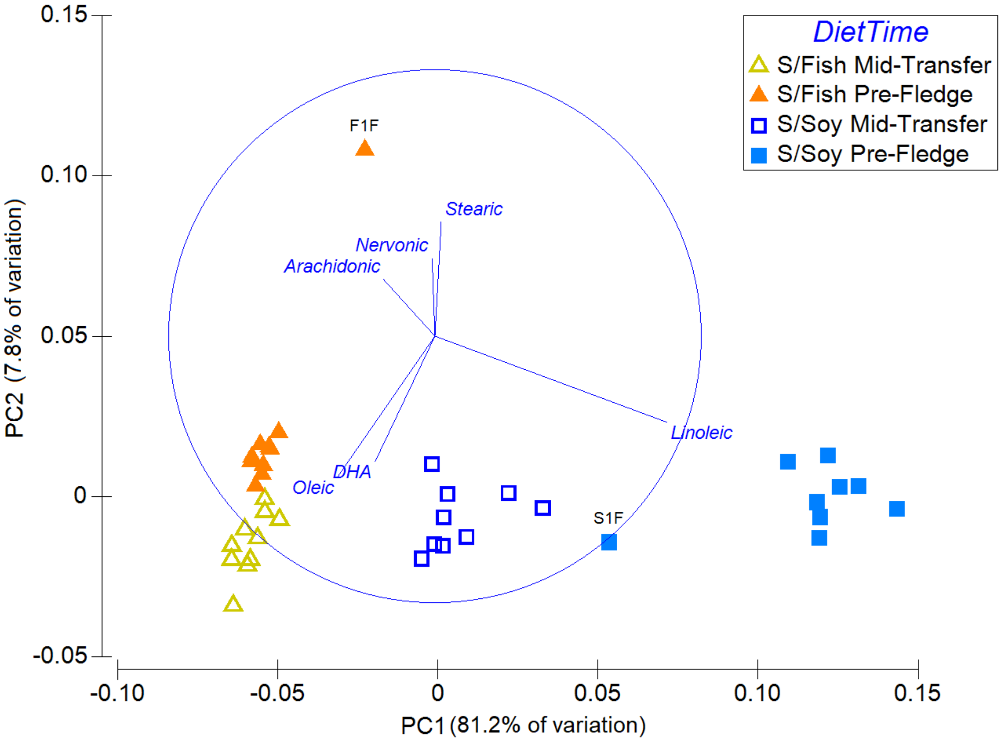


Figure 19 PCA plot of fluttering shearwater chick RBC fatty acid %, showing the change in the composition of membrane fatty acids 3-4 days after transfer and two weeks later near fledging and comparing two different dietary groups S/Soy n=9 and S/Fish n=10.

Outlier S1 is a sick chick and it's pre-fledging sample is labelled (S1F). Oulier F1 is a chick that is distinctly high in nervonic acid and it's pre-fledging sample is labelled (F1F).

The PCA plot (Figure 19) shows strong separation in the blood sample fatty acid profiles between diets. S/Fish-fed chicks varied slightly with time, while S/Soy fed chicks showed strong differences over time.

The vector overlays in Figure 19 indicate the fatty acids driving the differences in the PCA plot. Samples from birds fed fish oil showed higher levels of ARAC, OLE, DHA and NERV fatty acids, and low levels of LIN fatty acids. The birds fed soya oil showed the reverse results, with the difference being much more pronounced after two weeks. There was one notable outlier in the pre-fledge S/Fish (Chick F1F) (Figure 19). There is no clear reason in the transfer notes or clinical history as to why this chick would be an outlier. F1F showed significantly elevated levels of NERV in the pre-fledging sample. This chick has since returned to nest at the colony (Figure 20).

Of the 26 chicks that have returned to the colony, n=6 were retained in the PCA sampling group, (n=5 in the S/Fish and n=1 in the S/Soy dietary groups), (Figure 20).

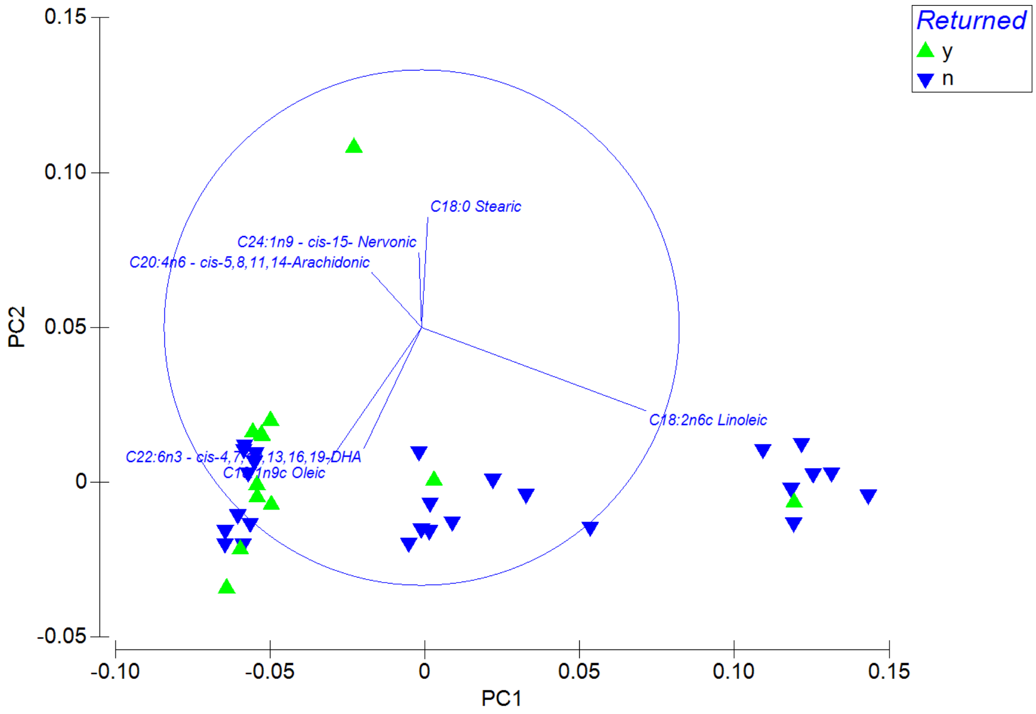


Figure 20 Principal component analysis plot showing the RBC phospholipid fatty acid ratios of chicks that have since returned to the colony n=11 (green Δ). Vector overlays show the fatty acids with the most difference between the groups.

2.4.12 FS PERMANOVA of RBC fatty acid profiles.

There was strong evidence of significant differences in the RBC fatty acid profiles between diets, and these differences change over time (Pseudo-*F* = 76.93, *p* = 0.0001), Table 10.

Table 10 Fluttering shearwater red blood cell fatty acid PERMANOVA table of results comparing the S/Soy and S/Fish dietary groups over time and between individuals.

Source	Df	SS	MS	Pseudo-F	P(perm)	perms
Diet	1	0.126	0.126	174.45	0.0001	9896
Time	1	0.040	0.040	108.43	0.0001	9939
Bird (Diet)	17	0.013	0.001	2.0341	0.0003	9858
Diet x Time	1	0.028	0.028	76.93	0.0001	9944
Res	15	0.005	0.000			
Total	35	0.215				

Unlike the GFP, the FS showed some consistent variation associated with individual birds (*p* =0.0003) indicating that to some degree, a bird's fatty acid profile after two weeks was more similar to its previous reading compared to a random bird. Including the individual *Bird* term in the model means there is very little replication at the lowest level, therefore the estimates of residual variation should be treated with caution.

Table 11 Estimates of component variation in fluttering shearwaters red blood cell phospholipid fatty acid analysis between 2 dietary groups S/Soy and M/Fish, over time and between individual birds.

Source	Estimate	Sq.root
S(Diet)	0.00742	0.08611
S(Time)	0.00232	0.04813
V(Bird (Diet))	0.00020	0.01417
S(Diet x Time)	0.00327	0.05723
V(Res)	0.00037	0.01911

The components of variation show that the diet fed, the time between sampling events, and the interaction between these two parameters have the biggest impact on a chick's fatty acid profiles, while random (residual) variation and variation between individuals have smaller impacts, (Table 11).

2.4.13 FS individual RBC fatty acid comparisons at different stages of the transfer

There was good evidence for differences in the means of individual fatty acids between dietary groups and within individuals over the period of the feeding trial, (Figure 21, Figure 22-26).

Similar to GFP ALIN was only present in small amounts, however, in FS there was a small yet significant increase in ALIN proportions on the S/Soy diet towards fledging, compared to S/Fish fed chicks and in S/Soy fed individuals, (Figure 21, Figure 22-26).

Relative to GFP, CAP was present at very low proportions in FS and decreased over time on both diets (Figure 21, Figure 22-26).

OLE and LIN in FS followed a similar trend as in GFP, with a significant difference in OLE and LIN proportions between all dietary groups, over time and between individuals (Figure 21, Figure 22-26). OLE was higher at mid-transfer in the S/Fish dietary group (mean % =20.93%) compared to (17.41%) on S/Soy, which was surprising, because by this stage the different diets had only been fed for 3-4 days. Over the next two weeks the proportion of RBC fatty acids that contained OA decreased significantly in both groups, by only a small margin in birds fed the S/Fish diet (to 19.23%) while there was a marked decrease in the birds fed the S/Soy diet was more pronounced (to 13.7%) (Figure 21, Figure 22-24).

LIN proportions increased in birds fed the soya oil diet, cumulating from 8.3% to 18.13% at pre-fledge blood sample. In birds supplemented with fish oil, the LA proportion did not exceed 2.7%, and decreased slightly from transfer to fledge, reaching 2.21% (Figure 21, Figure 22-26).

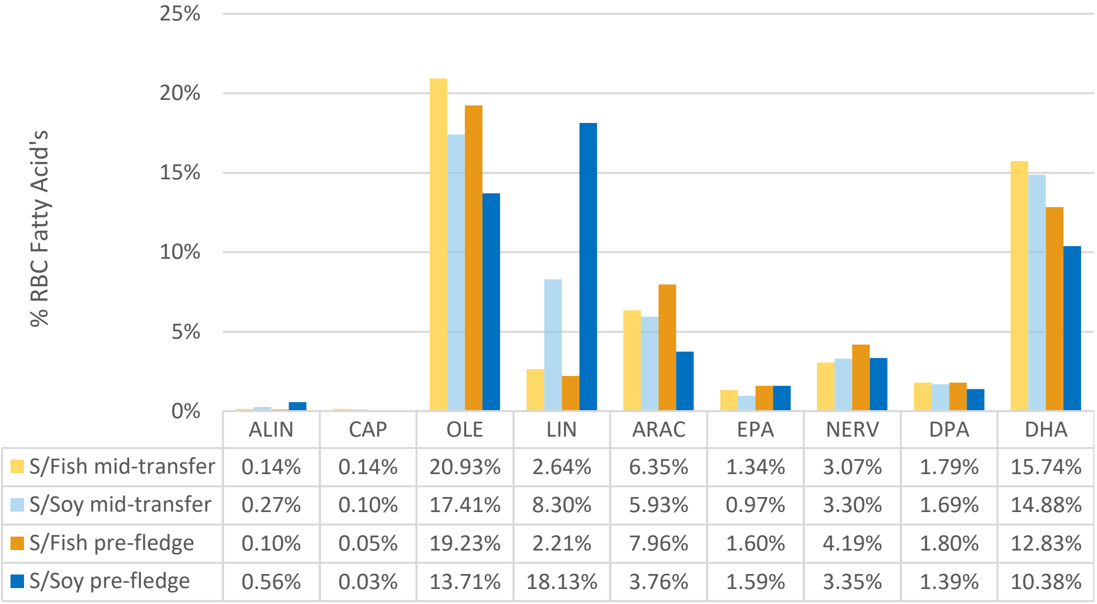
ARAC followed the same pattern in both FS and GFP, there was no difference between dietary groups at mid-transfer, but by the pre-fledging blood sample, the fatty acid proportions diverged significantly with ARAC proportions in chicks increasing in the S/Fish and decreasing in the S/Soy dietary groups (Figure 21, Figure 22-24). Chicks had marked individual variation in ARAC proportions between the dietary groups, increasing in birds fed S/Fish and decreasing in birds fed S/Soy (Figure 24-26).

The proportion of RBC phospholipid EPA was not significantly different between dietary groups, or individuals on each diet (Figure 21, Figure 22-26).

There was evidence of a difference in NERV proportions between both diets and individuals (Figure 21, Figure 22-26). The proportion of NERV increased significantly in birds fed the S/Fish diet by fledging but stayed unchanged in the S/Soy group (Figure 21, Figure 22, Figure 23). There was notable variation in the NERV proportions between individuals on the S/Fish diet, as seen by the notable outlier F1F in the PCA graph (Figure 19 and Figure 24).

DPA was present at relatively similar small proportions between dietary groups but dropped significantly by pre-fledging in birds in the S/Soy diet group. There was significant variation in DPA proportions within birds in the S/Soy diet group compared to birds fed the S/Fish diet, (Figure 21, Figure 22-26).

The average mid- transfer proportions of DHA were not different between the dietary groups (near 15%), however, DHA proportions in both dietary groups and individual chicks decreased significantly over time. By the pre-fledge blood sampling, the average proportions of DHA in birds on the S/Fish diet decreased to 12.83% and in birds on the S/Soy diet to 10.38% (Figure 21, Figure 22-26). Significant decreases were present in individual birds on both diets (Figure 24, Figure 25). DHA was depleted significantly faster in the S/Soy dietary group (Figure 26).



	ALIN	CAP	OLE	LIN	ARAC	EPA	NERV	DPA	DHA
■ S/Fish mid-transfer	0.14%	0.14%	20.93%	2.64%	6.35%	1.34%	3.07%	1.79%	15.74%
■ S/Soy mid-transfer	0.27%	0.10%	17.41%	8.30%	5.93%	0.97%	3.30%	1.69%	14.88%
■ S/Fish pre-fledge	0.10%	0.05%	19.23%	2.21%	7.96%	1.60%	4.19%	1.80%	12.83%
■ S/Soy pre-fledge	0.56%	0.03%	13.71%	18.13%	3.76%	1.59%	3.35%	1.39%	10.38%

Figure 21 Bar chart showing the comparison of mean RBC phospholipid fatty acid % between individual fatty acids on each diet at mid-transfer and fledge

Differences in RBC fatty acids between artificial diets.

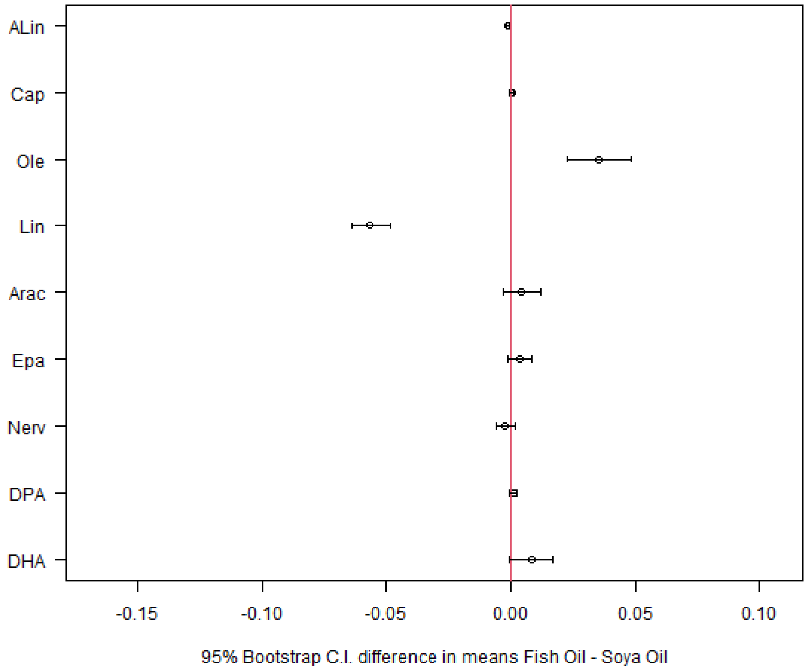


Figure 22 Mid-transfer differences between chicks fed the S/Fish or S/Soy diet. *

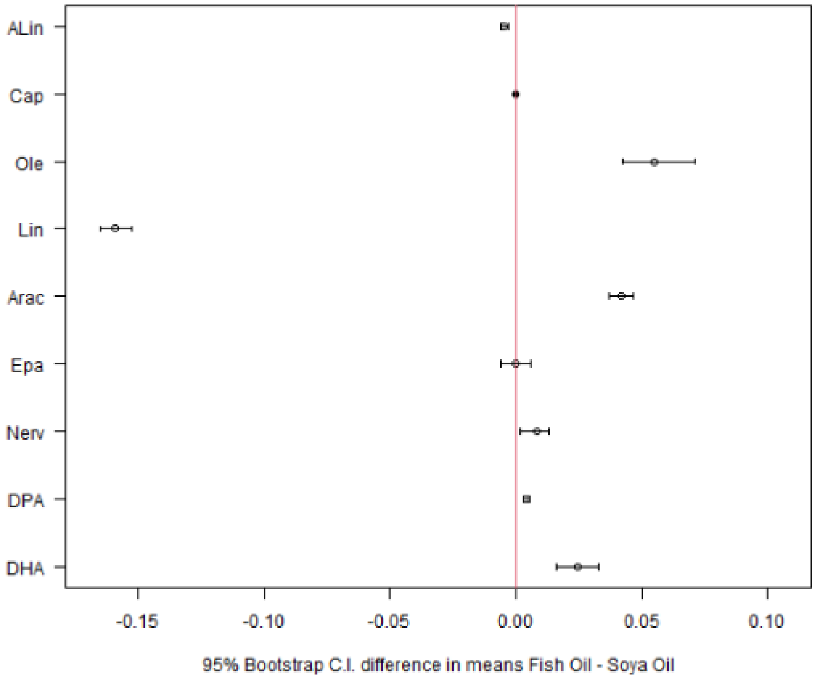


Figure 23 Pre-fledge differences in chicks fed the S/Fish or S/Soy diet. *

**Changes in mean % of nine RBC phospholipid fatty acids in fluttering shearwater chicks, graphed by bootstrap confidence intervals. Error bar represents the 95% confidence interval.*

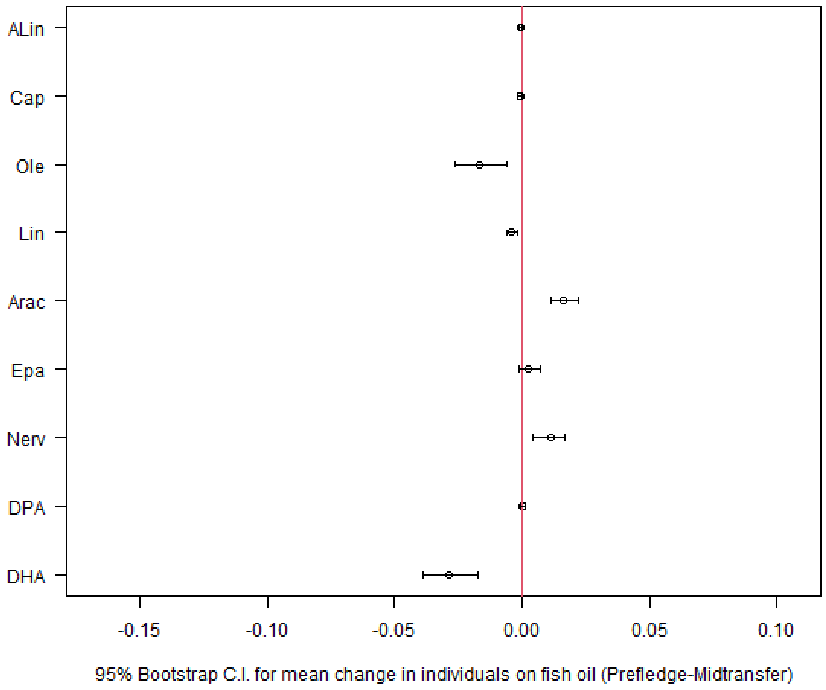


Figure 24 Changes within chicks fed the S/Fish diet from mid-transfer to pre-fledge. *

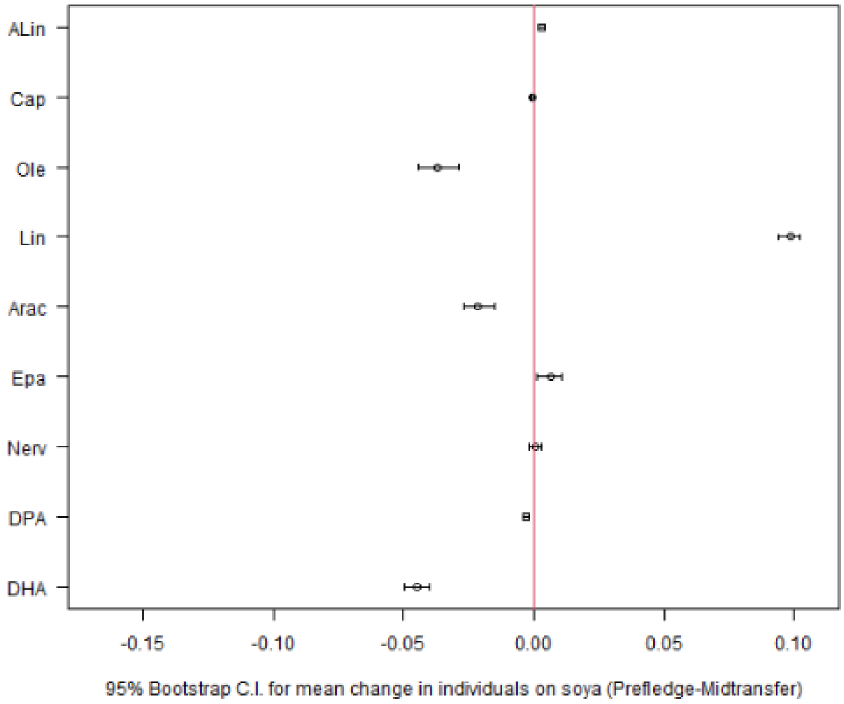


Figure 25 Changes within chicks fed the S/Soy diet from mid-transfer to pre-fledge. *

**Changes in mean % of nine RBC phospholipid fatty acids in fluttering shearwater chicks, graphed by bootstrap confidence intervals. Error bar represents the 95% confidence interval.*

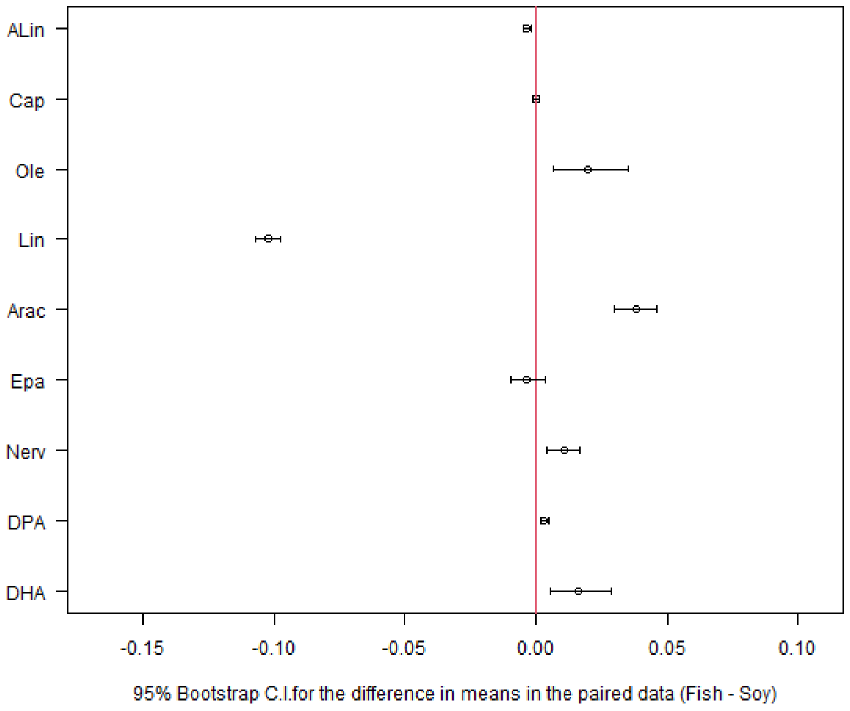


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Figure 26 Differences in individual chicks, between dietary groups S/Fish and S/Soy, over the transfer from mid-transfer to pre-fledge*

**Changes in mean % of nine RBC phospholipid fatty acids in fluttering shearwater chicks, graphed by bootstrap confidence intervals. Error bar represents the 95% confidence interval.*

2.4.14 Artificial dietary oil fatty acid analysis

Analysis of the fat in the three artificial diets illustrates a greater percentage of LIN and ALIN in soya-oil based diets and a lower PUFA percentages of (ARAC, EPA, DPA, DHA and NERV) relative to fish oil supplemented diets (Table 12).

Table 12 Fatty acid proportions in the oils of the three artificial diets, S/Soy, M/Fish and S/Fish.

Fatty acid analysis.	S/Soy	M/Fish	S/Fish
C6:0 Caproic	0.00%	0.00%	0.10%
C8:0 Caprylic	0.00%	0.00%	0.00%
C10:0 Capric	0.00%	0.11%	0.10%
C11:0 Undecanoic acid	0.00%	0.00%	0.00%
C12:0 Lauric	0.08%	0.15%	0.12%
C13:0 Tridecanoic	0.00%	0.08%	0.13%
C14:0 Myristic	5.24%	7.09%	6.03%
C14:1n5 - cis-9-Myristoleic	0.08%	0.07%	0.20%
C15:1n5 - cis-10-Pentadecenoic	0.00%	0.00%	0.10%
C16:0 Palmitic	13.06%	20.40%	20.50%
C16:1n7 - cis-9-Palmitoleic	3.95%	9.73%	6.82%
C17:0 Margaric	0.16%	0.91%	0.78%
C17:1n7 - cis-10-Heptadecenoic	0.00%	0.00%	0.00%
C18:0 Stearic	3.15%	4.94%	4.37%
C18:1n9t Elaidic	0.00%	0.18%	0.28%
C18:1n7t Vaccenic	0.00%	0.08%	0.12%
C18:1n9c Oleic	15.00%	17.31%	23.84%
C18:1n7c Vaccenic	1.53%	3.17%	3.29%
C18:2n6t Linolelaidic	0.00%	0.00%	0.00%
C18:2n6c Linoleic	31.21%	4.33%	2.25%
C18:3n6 - cis-6,9,12-Gamma linolenic	0.00%	0.22%	0.18%
C18:3n3 - cis-9,12,15-Alpha linolenic	4.76%	1.34%	1.08%
C20:0 Arachidic	0.32%	0.33%	0.27%
C20:1n9 - cis-11-Eicosenoic	9.35%	3.63%	9.86%
C21:0 Heneicosanoic	0.00%	0.00%	0.00%
C20:2n6 - cis-11,14-Eicosadienoic	0.16%	0.23%	0.36%
C22:0 Behenic	0.24%	0.19%	0.22%
C20:3n6 - cis-8,11,14-Eicosatrienoic	0.00%	0.15%	0.16%
C22:1n9 - cis-13-Erucic	0.73%	0.61%	1.22%
C20:3n3 - cis-11,14,17-Eicosatrienoic	0.16%	0.27%	0.36%
C20:4n6 - cis-5,8,11,14-Arachidonic	0.24%	1.34%	1.03%
C23:0 Tricosanoic	0.08%	0.00%	0.10%
C22:2n6 - cis-13,16-Docosadienoic	0.00%	0.00%	0.10%
C24:0 Lignoceric	0.08%	0.11%	0.10%
C20:5n3 - cis-5,8,11,14,17-EPA	5.00%	8.93%	7.27%
C24:1n9 - cis-15- Nervonic	0.40%	0.81%	1.14%
C22:5n3 - cis-7,10,13,16,19-DPA	0.48%	1.65%	1.42%
C22:6n3 - cis-4,7,10,13,16,19-DHA	4.52%	11.64%	12.12%

2.4.15 Post mortem results.

A total of ten GFP and six FS died during the study period, and full post mortem examinations, including histology, were undertaken on 13 of these birds (n=7 GFP; n=6 FS) (Table 13).

Table 13 Causes of death during translocation of grey-faced petrels and fluttering shearwaters.

Cause of Death	Grey-faced petrels (n=76)		Fluttering shearwaters (n=80)	
	S/Soy Diet n=40	M/Fish Diet n=26	S/Soy Diet n=51	S/Fish Diet n=19
Heat stress	0	0	6*	
Visceral gout and infection (myocardial necrosis/ bacterial enteritis)	1	0	0	0
Oesophageal laceration and infection	1	0	0	0
Predation (hawk)	2	0	0	0
Visceral gout and trauma	1^	1	0	0
Aspiration	1	0	0	0
Environmental	0	1	0	0
Missing – Unknown	0	2	0	0

* Chicks died on day of transfer. ^ Fed a salmon slurry (SAM) diet during the hospital stay.

GFP post mortem findings

All chicks necropsied had abundant subcutaneous, coelomic and epicardial adipose reserves. Table 13 summarises the ten deaths in GFP colony, which included n=2 hawk predations, n=2 chicks that strayed from the burrow early with wing lengths <270mm (which are considered unlikely to have fledged successfully at this size), as well as n=1 chick fledged early in a storm and washed up on the beach the next day. N=3 sustained injuries possibly related to hand feeding, n=1 regurgitated and aspirated in the nest box, n=1 oesophageal laceration with a secondary infection that may have been caused by crop tubing trauma early in the first days of translocation, and n=1 was found in the nest box with bilateral tibiotarsal fractures. The leg fractures healed well with treatment off-site at an off-site clinic, however, the chick died 11 days after return to the colony due to complications from severe visceral gout. Head trauma and visceral gout occurred in n=1 chick.

Three GFP chicks showed renal gout. Visceral gout was found in one chick on each diet- M/Fish, S/Soy, and one fed on a modified hospital diet that consisted of pureed frozen salmon, water, Mazuri® vitamin supplement, with no oils added (SAM) and the S/Soy diet while at the colony. All of the affected chicks were on site for > 11 days, while chicks that died without gout were only present for shorter periods ranging from 5, 8 and 9 days, (causes of death were regurgitation/aspiration pneumonia, oesophageal tear and secondary infection, and predation with per-acute (terminal) septicaemia respectively (Table 13).

Pathology of three grey-faced petrel chicks that developed renal complications.

Chick #40 was in the S/Soy dietary group and died after 14 days on-site, with post mortem findings of visceral gout, myocardial necrosis and bacterial enteritis (Figure 27). Gross post mortem examination showed chalky white uric acid precipitates diffusely over the pericardium, air sacs, coelomic musculature and serosal surfaces. Histology of the kidneys showed multifocal tophi of uric acid crystals within the remnants of renal tubules associated with basophilic mineralisation. Uric acid tophi were also present in the spleen and lung. In the heart, there were small haemorrhages in the cardiac muscle, and occasional areas of cardiac muscle degeneration. Throughout the intestines, there were small multifocal areas of acute inflammatory cells, associated with small aggregations of cocci. No other abnormalities were detected on histology.

Figure 27 Gross necropsy images of visceral gout in a grey-faced petrel chick #40 fed the S/Soy diet.

Pictures (Left) White chalky precipitates over the pericardium and serosal surface of the liver, air sacs and coelomic musculature. Abundant coelomic adipose reserves. (Upper Middle) Uric acid deposition in the epicardium with pericardial effusion. (Lower middle) Coalescing uric acid plaques on the lung. (Right) Uric acid precipitation overt the serosal surfaced of the intestine.

Chick #64 was fed the M/Fish diet and was found dead in its burrow, 16 days after transfer, with another chick sitting on top of it. Gross necropsy showed a small compression fracture on the posterior cranial fossa with cervico-cerebellar haemorrhage. Uric acid crystals were present at gross necropsy over the pericardium and kidneys. Histology of the kidneys showed occasional renal tubular cells that contained basophilic mineralised material and occasional degeneration of tubular epithelial cells. Proteinaceous casts were present in some tubular lumen and crystalline casts in others. There was no cellular response to these changes. The spleen showed small irregular areas of scattered necrosis and fibrin deposition, with the occasional suggestions of crystalline tophi but no associated organisms visible. The intestines contained ingesta and myriad bacteria but the villi showed no abnormalities. No other abnormalities were detected on histology. The primary cause of death in #64 is suspected to be head trauma either from the conspecific or from rearing back in the artificial box too quickly, however, death from renal compromise cannot be ruled out as visceral gout was developing in the kidneys, spleen and pericardium. # 64 was very close to fledging with a wing length 305mm and weight of 504.9g and only a small amount of down present on the neck.

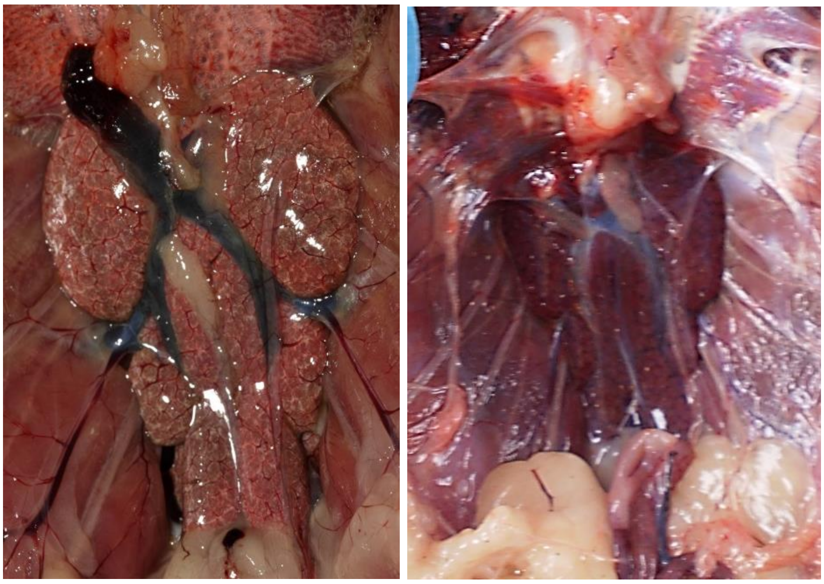

Figure 28 Gross necropsy pictures of two grey-faced petrel chicks fed M/Fish diet.

(Left) Chick #64 shows white chalky uric acid deposition throughout the parenchyma. (Right) Chick #21 kidney showing normal kidneys on gross necropsy (scattered with a few grains of sand).

Chick #70 was in the S/Soy dietary group and was found in its burrow with bilateral tibiotarsal fractures six days after transfer. It was transferred to Massey University where it was treated for 31 days and fed a hospital diet (SAM), during this time it had several anaesthetics for medical procedures where supplementary fluids were given. The chick was returned to the colony and fed onsite with the S/Soy diet EOD for 11 days. During this time, the chick maintained weight and the wings were growing, until the day before death where it was noted to be lethargic. At necropsy, the chick weighed 480g, was in excellent body condition and had plentiful fat reserves. Visceral gout was affecting the kidneys and the serosal surfaces of the liver and heart with widespread metastatic calcification in the glands of the proventriculus, cardiomyofibres of the heart and lung interstitium. The tibiotarsal fractures had callused well and were nearly completely healed. Gram-negative bacterial enteritis was present, that was not cultured but suspected to be Ecoli, salmonella or yersinia.

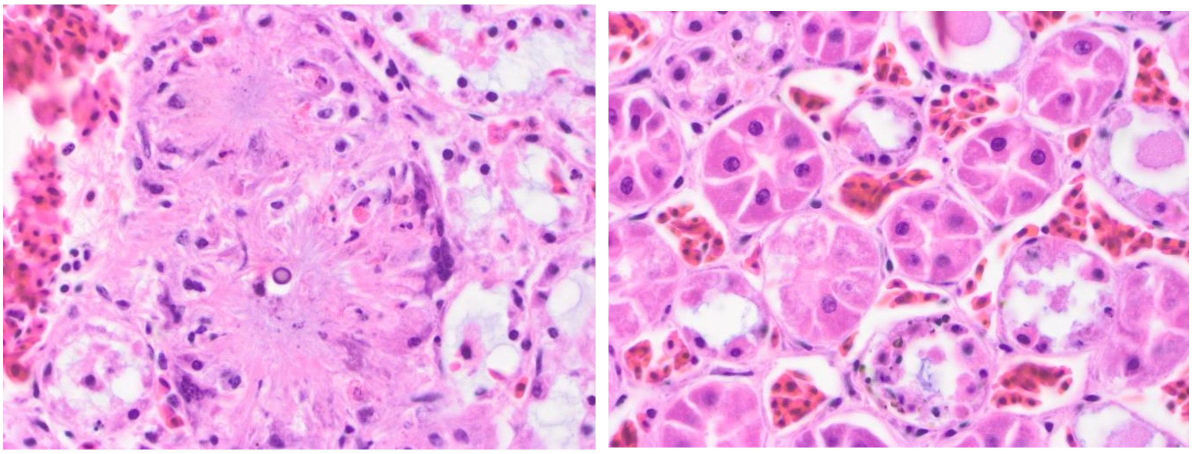

Figure 29 Renal histology of #70 grey-faced petrel chick with visceral gout. The chick was fed two different diets the S/Soy diet and a hospital diet of pureed salmon SAM.

(Left) x100, shows uric acid crystal deposition. (Right) shows varying stages of renal tubular necrosis. X100 Hematoxylin and eosin stained.

FS post mortem findings

In the FS trial, all 80 chicks were boxed up early in the morning on the day of transfer and travelled by boat to the helicopter site, however, due to helicopter delays the transfer took several hours longer than anticipated. One chick died during transit, five chicks regurgitated on arrival and were given oral fluids and died within 24 hours of arrival. All 6 chicks died of heat stress and were amongst the heaviest chicks in the group. No FS chicks died during the dietary trial period.

2.5 Discussion

2.5.1 Summary of key findings

This study assessed the effect of artificial diets fed during two translocations of seabird chicks by comparing changes in RBC fatty acid ratios, fledging parameters, general health, necropsy results and return rates to the colony. The key findings from the study were that the S/Soy diet fledged GFP chicks at significantly higher weights than fish-oil fed chicks, while the weight of FS was not affected. Wing length and emergence were similar between groups. Over a 2-3 week feeding period, supplementing translocation diets with fish oil in chicks of both species studied improved or maintained fledging RBC fatty acid ratios of DHA, DPA, and ARAC to levels closer to their natural diet. NERV proportions were increased in FS fed fish oil but showed no effect in GFP. Feeding chicks the S/Soy diet provided lower DHA and ARAC than fish oil (S/Fish or M/Fish) and Wild diets and drastically increased the proportions of LIN and ALIN in RBC membranes. The changes in RBC membrane fatty acids support the hypothesis that seabirds fed fish oil will fledge with fortified fatty acid ratios that are closer to their natural diet, with higher proportions of very long-chain PUFA omega 3 and 6's, rather than needing to synthesize them from LIN or ALIN offered in the soya oil diet. A conversion that may not be efficient or possible in some seabirds. Return rates of FS to the colony nine years later have not been significantly affected by the variation in dietary oil, with fish-oil fed chicks returning at 40% compared to soya-oil fed chicks 34.6%. The return rate of GFP has not been assessed, so the effect on long-range foraging species remains unknown. A concern in the GFP group is that irrespective of the diet fed visceral gout occurred in both dietary groups and further investigation into the diets fed will be needed to identify if nutritional limitations have caused this condition.

2.5.2 Feeding trial fledging parameters

Weight gain on translocation diets.

S/Soy fed GFP chicks fledged at heavier weights than chicks fed the M/Fish diet. Heavier weights at fledging have the potential to be either beneficial or detrimental. Diving petrels fed the S/Soy diet fledged at higher weights, which correlated to higher survival to adulthood (Miskelly & Taylor, 2004), although this species does not produce proventricular oil. As translocation protocols have been refined, there is concern that in some species of petrels, which can fledge at weights heavier than adult birds (such as GFP, Chatham petrels (*Pterodroma axillaris*), Cook's petrels (*Pterodroma cookii*) and Pycroft's petrels (*P. pycrofti*) could have difficulty fledging and dispersing successfully. Therefore, translocations aim to feed chicks a diet that reduces weight gain to near parent weight before fledging (Gummer, Taylor, et al., 2014a; Gummer, Taylor, Collen, et al., 2014). The wild growth curve of GFP chicks shows substantial weight gain and loss, that peaks around 75-80 days of age. In a good season, well-provisioned chicks may be substantially heavier than adults (800-1100g) (G. Taylor per obs, cited by Gummer, Taylor, Colle, et al ., 2014). The volume and frequency of feeds given by parent birds reduce so that the chicks will fledge near-adult weights (Dunn, 2012). However, substantial individual variation in the weights of wild chicks exists and means that clear weight gain and loss patterns do not apply consistently in the wild. (Dunn, 2012). In a poor provisioning season, chicks that do not reach peak weight will continue to gain weight up until departure. Weight gains on the fish-oil supplemented diets were closer to the aims of the translocation than the soya oil supplemented diet in GFP chicks. Irrespective of the diet fed to GFP in this study, adipose tissue reserves were substantial in chicks necropsied during the GFP translocation studied.

In comparison to other studies, chicks fledging in higher weights is not unexpected on the S/Soy diet, as four[4] of the seven previous petrel translocations assessed in (Miskelly et al., 2009) fledged at significantly higher weights than compared to their natural parent fed colony. However, reports of translocations before 2009 found both GFP and FS fledging weights did not increase on the S/Soy diet relative to wild colonies (Miskelly et al., 2009). The ideal fledging weight for translocated chicks needs further assessment by reviewing the successful return rates to determine the relative risk of fledging at heavier weights.

2.5.3 Measures of growth

No difference in average wing growth or time-on-site was detected in either GFP or FS. Known growth curves in wild colonies can be highly variable due to the wide range of prey sources exploited, infrequent feeding of chicks (Puskic et al., 2019) and variation between seasons. In this study, unknown hatch dates were also a factor. Further assessment of growth morphometrics was not undertaken since chicks follow a typical growth pattern for Procellariiformes: the tarsus length, bill length and bill depth all reach an asymptote, so the chicks would already have been comparable sizes with adults in the 20 days before fledging

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[4] Fairy prion (*Pachyptila turtur; tītī* wainui), Pycroft’s petrel (*Pterodroma pycrofti*), Chatham petrel (*Pterodroma axillaris*; ranguru) and Hutton’s shearwater (*Puffinus huttoni*; Kaikōura tītī) all fledged at heavier weights than wild colonies, while fluttering shearwater (*Puffinus gavia*; pakahā), Common diving petrel (*Pelecanoides urinatrix*; kuaka), and grey-faced petrel (*Pterodroma macroptera gouldi*; kuia) did not. Chatham Island petrels(*Magenta petrel; Pterodroma magenta*; tāiko) numbers were too small to be statistically significant. (Miskelly et al., 2009)

(Dunn, 2012). Sub-clinical changes due to diet can be difficult to detect, therefore the preference in this study was to compare RBC phospholipid membrane fatty acid proportions between dietary groups. This is a standard technique to assess very long-chain PUFA (omega 3 and omega 6) deposition in functional cells and tissues, including DHA proportions in the brain, heart and retinal tissue of mammals. (Bradbury, 2011). Measuring RBC fatty acids to assess the impact of dietary oils has been used in several species of birds including cockatiels *Nymphicus hollandicus* (Heinze et al., 2012), king penguins *Aptenodytes patagonicus*, pigeons (*no species defined*) (Fayolle et al., 2000; Watts. C & Wheeler. K, 1978) and chickens *Gallus gallus*, (Kartikasari, 2009). This study is the first to assess RBC membrane fatty acid ratios in GFP and FS and the effects of dietary oils of membrane phospholipid proportions over time are evident.

2.5.4 Fledging parameters

Fledging statistics of translocations are often compared by the numbers of presumed fledges, fledging weight, wing length and time-on-site, however, these parameters can be complicated to record accurately, and can be a limited indicator of the success of a diet during a transfer, due to the multiple variables that must be considered. When the chicks disappear from their burrows, there is no guarantee the chicks fledged overnight, as some may have wandered into bushes to fledge at a later date, and many will visit other burrows before fledging (both natural and artificial). On some days, large numbers of chicks fledge together despite a difference in ages and growth (pers.comm Ward-Smith, 2010) and it is unclear if this behaviour is determined by weather or behavioural cues within the colony. The weight of chicks before fledging can vary widely based on when the chick was last fed and if the chick has been regurgitating in the days prior in preparation for fledging. (Gummer, Taylor, Collen, et al., 2014). Translocation fledging rates are often reported by counting chicks as likely or unlikely fledges. Comparisons of translocation fledging percentages and return rates can easily be affected by the criteria that constitute ‘unlikely fledge’ at the time of transfer, especially when considering return rates of FS where only small numbers of chicks were fed the fish oil diet.

Confidence in the accuracy of fledging statistics in this study has increased by the intensive monitoring undertaken over the following decade and by the number of FS chicks that have returned given a less than optimal start. The S/Fish group included a chick that returned having fledged well below recommended fledging weight. In subsequent transfers, lighter chicks have been considered to have higher chances of returning than previously. The S/Soy return group included a chick that regurgitated in the transfer box, which is interesting because all other chicks that regurgitated during this transfer died of heat stress, so it is valuable to know the chick fully recovered. In the S/Soy group (n=2) chicks have returned that disappeared with < 50% and <20% down-cover and were thought to be hiding out in nearby vegetation. 100% (n=3/3) of chicks that screened positive for low coccidia burdens have since returned and this is comprised of (n=2) S/Fish and (n=1) S/Soy fed chicks. Both fish oil and soya oil rich in ALIN and LIN have proven health benefits in poultry affected with coccidia, with fish oil proven to be more effective (Pike, 1999). No effect of diet has been noted in return rates of FS. The numbers of fish oil translocated chicks were small and further assessments of subsequent translocation return rates is recommended.

2.5.5 Return rates

In 2021, the FS chicks that had been fed fish oil returned at 5.4% higher rate, a difference that is not significant between diets. The monitoring on this colony has been exceptionally thorough with monthly checks since 2012. Given the small numbers fed different diets some fluctuation is still likely. Findings from the Mana Island FS translocation have shown that some chicks may not return until 11 years after the transfer, so the total percentage return rate could still be changing up to 2023 (pers. comm Cotter, 2021).

The effect of dietary oils on return rate in longer strategy foraging species has not been established in this study, but further investigation in return rates of translocated chicks relative to diets fed and potential disease incidence during transfers would be highly beneficial.

2.5.6 RBC phospholipid fatty acid similarity to wild diets.

Similarities between species

Similar trends of RBC fatty acid changes occurred in both species in response to the inclusion of either fish oil or soya oil in the diet. Most notably when soya oil was fed to translocated individuals of either species, there were significant decreases of OLE, DHA and ARAC, while large proportions of LIN built up in RBC membranes, compared to all other dietary groups.

The significance of the changes soya oil produced in the RBC fatty acid ratios is best considered in the context of the importance of fatty acids in wild Procellariiformes diets. A consistent finding in wild Procellariiformes proventricular oil samples is that OLE is the most predominant fatty acid, while LIN is only found in very low or trace proportions (Connan et al., 2007; Hendriks et al., 2000). The effect of displacing OLE with LIN is unknown in seabirds, however, this study has shown that as LIN increased substantially in the S/Soy diet, there was a decrease in the relative proportions of very long-chain (>C20) PUFA, (DHA, DPA and ARAC). Depleting membrane reserves of very long-chain PUFA and replacing them with precursor fatty acids, may be beneficial, but only if it can be utilised.

Since seabirds have a marine-based diet rich in very long-chain PUFA it is unknown if seabirds are capable of using LIN and ALIN to effectively elongate and desaturate to form ARAC, as many obligate piscivorous species have lost this trait (Klasing, 1998). Even in avian species that benefit from the provision of LIN and ALIN, providing DHA and EPA has proven to be more effective as the conversion rate is rather low due to the competitive enzymes involved. (Koppenol et al., 2014), (Cherian, 2015).

In birds, nutrient deficiencies can present acutely or chronically and defects in nutrient supply during chick growth has the potential for permanent damage. (Klasing, 1998) Decreases in DHA on the S/Soy diet in GFP chicks and on both diets in FS trail is concerning and supplementation or correction should be considered.

PUFA-rich marine-based diets have many proven health benefits and may also play an important role in migration and thermoregulation (Hulbert & Abbott, 2012). Numerous mammalian, bird and fish studies have shown that n-3 PUFA stimulates cellular capacity for lipid oxidation in hepatocytes, cardiomyocytes and adipocytes (Maillet & Weber, 2007). In preparation for long migrations, the semipalmated sandpiper (*Calidris pusilla*) feeds on marine invertebrates rich in n-3 fatty acids DHA and EPA which are incorporated into muscle cell membranes, acting as molecular signals to prime flight muscles for extreme exercise, and to increase their oxidative capacity (Maillet & Weber, 2007). These findings have been replicated in bobwhite quails (Colinus virginianus) (Nagahuedi et al., 2009) and increased efficiency in muscle oxygen consumption and reduced muscle fatigue have been recorded in rats fed fish oil diets. (Peoples & McLennan, 2010). Pre-loading cell membranes with DHA by feeding fish oil may benefit chicks at fledging by improving the oxidative capacity of cells before the chicks learn to fly, forage and disperse.

Differences between species

Several species-specific differences occurred with fish and soya oil supplementation. We hypothesised EPA would have a similar trend to DHA, however, this was not the case in FS, where EPA showed no change in the S/Fish dietary group. In GFP there was a significant increase in EPA in fish-oil fed chicks compared to soya oil fed chicks, however, both artificial diets maintained levels proportionate to the Wild diet near fledging. As expected ALIN was only present in very small proportions in the Wild and mid-transfer diets and increased in FS chicks fed the S/Soy diet however, ALIN was unaffected in the GFP dietary trial.

The levels of NERV differed significantly between species. NERV is an omega n-9 PUFA that increased significantly in fish-oil fed FS chicks at fledge, yet appeared insignificant in the GFP. NERV values increased in the fish-oil fed group pre-fledge sample, but showed no change in S/Soy fed chicks or in any GFP, supporting the hypothesis that Procellariiformes with different foraging strategies are likely to have species-specific nutritional requirements. FS outlier F1F showed the greatest individual increase of all the chicks in NERV (3.1%), however, another individual within the FS group increased by (2.8%), so it is unlikely this outlier effect was unique to F1F but rather a reflection of the limitations of the small sample size. Given the RBC life-span, it is possible that the F1F chick was fed a dietary item in the wild colony that resulted in this spike. For example, Ibañez et al.(2021) noted increases in OLE and NERV in adult skua plasma near the end of chick-rearing, when the diet is more reliant on penguin eggs and marine foraging.

The effect of time on RBC phospholipid fatty acid turnover.

The variation in RBC fatty acids between individuals is likely to be highly influenced by fatty acid metabolism and the timing and composition of the last parent feed before transfer. Due to RBC turnover, the effect of changing diets on RCB fatty acid ratios will not be entirely captured in the 14-18 day sampling period. The maximum avian RBC life span is between 20-45 days and varies depending on the species (35 days for chickens and 45 for ducks) and is significantly shorter than in mammals (115 and 120 days for canine and human RBC) (Cital et al., 2016; Shaw et al., 2009). This shorter lifespan is considered to be associated with the increased metabolic rate and oxygen consumption of birds compared to mammals (Cital et al., 2016; Shaw et al., 2009). The RBC fatty acid ratios changed significantly over time during this trial. This is best illustrated in the examination of the GFP PCA graphs. In this analysis, outliers S3 and S4 were being fed the S/Soy diet for a mere four days longer, yet this increased their LIN proportions relative to the rest of the mid-transfer group and pre-fledging group. The timing of RBC turnover highlights the importance of the oils fed over the transfer period, as these oils will be affecting the chicks during the final stages of their growth period and in the critical weeks following fledging as the chicks learn to forage at sea. Conversely, it also means that any artificial diet deficiencies may be corrected by the fledglings within 45 days of the transition onto a wild diet, depending on prey availability and individual foraging success of each chick. In New Zealand the success of translocations has improved as pre-transfer chick selection size criteria for many species has been refined over the years, enabling shorter periods of feeding and reducing the risk of health complications that may be associated with hand-rearing.

Hangover effect of the diet

Variation in body weight due to the size of parent feeds at transfer affected the RBC fatty acid ratios of chicks in the dietary trial. It can be very difficult to detect the base (pre-feed) weight of petrels before translocations, due to the fluctuations in weight resulting from large, irregularly delivered parent meals (Gummer, Taylor, Collen, et al., 2014). GFP parent feeds can range from 40g to 160g in 24 hours, with the largest volume fed to a chick recorded as 225g, on a night, when both parents provisioned a chick nearly doubling the weight of the chick (Imber, 1976). Thus, transfer weights are commonly variable: for example, in the S/Soy dietary group, S10 weighed 910g and progressively lost weight over the translocation to fledging at 660g, while showing healthy wing growth, from 220mm to 315mm at fledging. These weights indicate that the chick had very recently been fed by a parent bird and a large proportion of the chick’s weight would be from an oily meal. While in comparison S4 the lightest chick in the group weighing only 510g and had most likely not been fed for several days. As some chicks have the potential to nearly double their body weight according to parental feeding patterns it follows, therefore, that a well-fed chick at transfer may have the equivalent of an extra week of artificial feeds when arriving on the translocation site, compared to chicks that had not been recently fed before transfer. Very large parent feeds before transfer are also likely to be disproportionally influencing RBC fatty acid ratios. This was evident in the heaviest chick in the S/Soy group’s mid-transfer and pre-fledge blood samples, which showed RBC fatty acid ratios more similar to those of fish oils supplemented and Wild dietary groups.

Based on these findings, selecting heavier chicks for transfer, provided they are still within the recommended wing lengths, is likely to be highly beneficial to translocation success, as a recent feed of wild proventricular oil will significantly change RBC fatty acid proportions, even on the S/Soy diet. Increasing proportions of DHA, DPA, ARAC, OLE and decreasing LIN in RBC’s fatty acid ratios will reflect fatty acid proportions that are more similar to the Wild-type diet. The effect of a recent parent feed before a transfer is likely to be more beneficial to long-strategy foragers (GFP) than daily foraging species (FS), simply due to the frequency of feeding and differing digestion time. The example of a uniquely high proportion of nervonic acid near fledging in a FS chick (F1F) is best explained by the consumption of an unusual wild prey item, suggesting that the effects of parental feeding can have a long hangover effect on PUFA proportions, even in daily foragers.

However, the benefits of an ample parent feed should also be considered with the higher risk of regurgitation during transport. In the translocation of FS in this trial, heavier chicks were more affected by heatstroke. Avoiding transferring chicks that have recently regurgitated is likely to be beneficial for the chick over the transfer, as will gentle handling since GFP chicks have a regurgitation reflex as a defence against predators (Foster et al., 2010; Swennen, 1974; Warham, 1977) and preserving this valuable food resource should be of paramount concern.

Food items from the parent feeds are still being digested throughout the GFP transfers. During seabird transfers, it is common for chicks to regurgitate food, and within two-weeks of transfer, the material regurgitated is still remarkably similar to regurgitation samples from Wild-fed chicks and distinctly different to the pale slurry of the artificial diets. Wild fed stomach contents often include bright orange proventricular oil which collects its reddish glow from astacin a pigment found in crustaceans, along with purple ingesta (potentially stained purple from squid ink or digestion), and with larger items retained in the gizzard (squid beaks, otoliths and krill exoskeletons).

2.5.7 Visceral gout.

Visceral gout secondary to renal failure has been found previously in GFP transfers (McInnes, 2007) and occurred in this GFP transfer on both diets. It was ultimately the cause of death for #70 chick that was fed the artificial diets for a prolonged period. Clinical signs of renal dysfunction prior to the deposition of gout crystals are difficult to detect in birds due to their uricotelic metabolism. If renal damage occurs due to either the translocation diets used or the management of fluid balance in the chicks, then this presents a high risk that chicks are fledging with compromised kidneys, which can impact their survival and future breeding potential. Deaths with signs of visceral gout in the GFP, but not in the FS, indicate that renal function is more easily compromised by the differences between wild and captive feeding, in Procellariiformes with long-foraging strategy species. Differences in either the dietary formulations used, the fluid balances, or even the frequency of feeding may all play a part.

Visceral gout is a condition where renal function is decreased to the point where the concentration of uric acid accumulates in the blood and body fluids. The uric acid accumulates to the point that it crosses a thermally regulated threshold for solubility and precipitates as calcium sodium urate crystals into a variety of locations, particularly the kidney and serous membranes of the liver, heart and air sacs, as observed in cases #40, #64 and #70. Visceral gout is an end-stage indicator of kidney damage that may have a number of potential causes including infectious, nutritional and toxic causes or a combination of several factors.

Nutritional or metabolic factors known to affect the kidneys include excess dietary calcium, low phosphorus, excessive sodium bicarbonate, vitamin A deficiency, toxic effects of excessive sodium and vitamin D3, excessive dietary protein and dehydration which may affect kidney function (Harrison & Lightfoot, 2006).

The timing of the deaths due to visceral gout indicate that renal failure most likely occurred at the new colony sites rather than before transfer to the translocation site, as in both cases the chicks had been at the transfer site for more than two weeks prior to death. Estimating the duration of renal failure and visceral gout in one GFP (#70) is complicated by a prolonged hospital treatment period.

Dehydration

Water deprivation can predispose to visceral gout (Harrison & Lightfoot, 2006). GFP are fed every other day to mimic long-distance foraging strategies in the wild and had a high prevalence of visceral gout, while FS have a daily feeding strategy and had no observed complications with visceral gout. Proventricular oil in long-strategy feeders is considered to be a dietary adaptation to allow long periods between feeds (Place et al., 1989). On average wild GFP chicks are provisioned once every 3.9 days, with the longest interval between feeds being 10 days (Imber, 1976). But it's uncertain what the long periods between meals means in terms of water balance and renal function. It seems counter-intuitive that a chick that is fed and hydrated more regularly should be more likely to develop renal failure, but it may be that the regular provisioning is disrupting the physiological mechanisms that allow the chicks to survive the long and irregular periods of parental feeding. Research into the water balance and renal physiology in wild long-distance foragers is highly recommended. Until emergence, the chicks usually stay in a cool burrow with minimal energy expenditure, so it may be possible that the additional exertion during handling and change in temperature is presenting a significant risk of dehydration.

A nutrient is considered deficient in the diet when the addition of more of the nutrient improves growth, reproduction, or general fitness or relieves some pathological condition. (Klasing, 1998). Artificial diets high in protein, sodium, vitamin D3 or calcium and/or low phosphorus and vitamin A present a potential dietary risk of developing visceral gout. Seabird diets are naturally high in protein, vitamin A and sodium relative to other species, however, the exact requirements remain unknown. Until more is known replicating findings in the wild diet is the safest approach to potential health complications. High arachidonic acid has been implicated in chronic renal disease, due to the production of prostaglandins and thromboxane, while DHA and EPA are recommended as a treatment for renal disease due to their renal protective properties(Harrison & Lightfoot, 2006).

2.5.8 Limitations of the research

Seasonal availability is a confounding factor in interpreting the nutrient value of wild diets. The year of the GFP trial was a La Niña year, which was an especially good year for the control colony (pers.comm Taylor, 2010). In other species of Procellariiformes improved fledging success has been linked to interannual variations in the availability of lipid-rich prey items (Connan et al., 2008). This study only covers the last three weeks of fledging, so the findings are only applicable to a 'finishing diet', while different life stages are likely to have different dietary requirements.

2.6 Summary

Feeding diets that are fish-oil based will potentially fledge chicks with RBC phospholipid fatty acid compositions that more closely resemble wild colony diets in DHA, DPA, ARAC and OLE. S/Soy dietary groups in both species had significantly increased LIN and significantly decreased OLE, DHA, DPA and ARAC, by fledging. EPA levels were relatively neutral in FS and levels did not change with the oils fed, while EPA increased slightly with fish oil supplementation in GFP chicks. Even though soya oil will dramatically change the RBC phospholipid membrane PUFA proportions, there was no detrimental effect on the success of FS return rates to Matiu/Somes Island. This limited effect may be due to the hangover effect of parental feeding and the likelihood that birds quickly re-establish PUFA proportions once foraging for themselves. However, there is also no data to assess what happens to the chicks at sea following fledging. Dietary fat content affects RBC fatty acid ratios even during a short feeding period. The ability of piscivorous seabirds to elongate and desaturate C18 plant-based fatty acids (LIN and ALIN) is unknown. Many obligate carnivores and piscivores amongst other species have lost the ability to synthesise essential fatty acids from plant-based oils, particularly arachidonic acid. Until further research is conducted a prudent diet would include ARAC and DHA supplementation (Klasing, 1998). Gout is a concern in GFP chicks and further research into the diets used in GFP is required.

Chapter Three

Nutritional composition of artificial hand-rearing diet compared to wild diets of *Pterodroma* and *Puffinus* petrel species.

3

3.1 Abstract

Protecting seabirds is a global conservation priority, considering that 29% of seabird species are threatened (Borrelle et al., 2018). Colony translocation of burrow nesting seabirds is a successful management strategy with far-reaching benefits for seabird conservation. Pivotal to the success of a seabird translocation is feeding a suitable artificial diet to pre-fledging chicks. However, the nutritional requirements of burrow nesting seabirds are currently unknown, making the formation of balanced artificial diets difficult. Studies investigating wild diets focus on the foraging range and prey items consumed rather than considering the nutritional components required to replicate a balanced chick diet. A diet of tinned sardines in soya oil (Brunswick™) (S/Soy) has been successfully fed to various species of burrow nesting petrels in New Zealand as a finishing diet. However, given the diversity of species and their foraging niches and prey selection, the 'one-size-fits-all approach is unlikely to meet the needs of all these species. This study compares the wild diet of three New Zealand burrow nesting seabirds with the current translocation diet S/Soy and two alternative artificial diets with fish oil added. The three species of petrel investigated represent three distinctly different foraging strategies: Chatham petrels (*Pterodroma axillaris;* ranguru) (CP), fluttering shearwaters (*Puffinus gavia;* pakahā) (FS), and GFP (*Pterodroma macroptera gouldi; kuia*) (GFP). The nutrient composition and fatty acid ratios of proventricular samples for each species were analysed and compared to the three different translocation diets. The effect of sampling methods on nutrient analysis was assessed by comparing results from proventricular flushing (PVF) and spontaneous regurgitation samples (REG).

Results showed that GFP diets had high variation in nutritional composition and the sampling method significantly affected results with REG samples demonstrating higher fat content. Ash content was highly variable in all species, particularly samples collected by PVF and increased the variation reported in proximate analysis results. Irrespective of the sampling method, all species' differences were widely apparent, with species with a short-foraging strategy showing higher protein and lower fat content than long foraging strategies. Each species showed a unique fatty acid profile. The fatty acid proportions of proventricular samples were not significantly affected by sampling methods but showed diversity between species and translocation diets.

The translocation group fed S/Soy had significantly higher linoleic acid C18:2n6 (LIN) and alpha-linolenic acid C18:3n3 (ALIN) proportions than any other group distinctly setting it apart from all other wild diets and fish oil supplemented translocation diets. Docosahexaenoic acid C22:6n3 (DHA) and arachidonic acid C20:4n6 (ARAC) were lower in the S/Soy diet than in fish oil supplemented diets and in both the GFP and FS wild dietary groups. Oleic acid C18:1n9 (OLE) and palmitic acid C16:0 (PAL) were predominant fatty acids in the wild type diets investigated and showed species-specific differences. No artificial diet provided sufficient nervonic acid C24:1n9 (NERV) to reach levels in wild diets, with a Brunswick™ tinned sardines in fish oil (S/Fish) being the closest alternative. The CP wild diet samples were uniquely high in (NERV) and its n-9 precursors (OLE, eicosenoic acid C20:1n9 and erucic acid C22:1n9). While unexpectedly, CP samples were low in DHA, docosapentaenoic acid C22:5n3 (DPA), eicosapentaenoic acid C20:5n3 (EPA) and ARAC levels in comparison to other species and artificial diets. Translocation diets based on tinned sardines with supplemented fish oil shared the closest similarity to the wild diet of all species studied. Given the importance of long-chain polyunsaturated fatty acids (PUFA) diets in chicks' health and development, a prudent diet would include supplementation with DHA, ARAC, and NERV fortified oils. The volume and proportion of fat-fed in translocation diets need further investigation, carefully considering the sampling methods used to determine normal fat levels within the diet.

Keywords

Proventricular flushing, regurgitation, Chatham petrels (*Pterodroma axillaris*), fluttering shearwaters (*Puffinus gavia*) and grey-faced petrels (*Pterodroma macroptera gouldi*), proximate analysis, C22:5n3 Docosapentaenoic acid (DHA) nervonic acid C24:1n9 (NERV), linoleic acid C18:2n6 (LIN), fish oil and soya oil.

3.2 Introduction

Translocation of burrow nesting petrel chicks is a commonly utilised conservation tool in New Zealand. This technique relies on the highly philopatric nature of these species, which are believed to imprint on their natal colony when they first emerge from their burrows (Gummer *et al.*, 2014). Chicks intended for translocation are left with their parents from hatching and only moved close to emergence from the burrow at around 5 weeks of age, to ensure they gain as many behavioural and nutritional benefits from their parents as possible before transfer. Therefore, translocation diets are only intended to be finishing diets and are only fed to chicks aged within 5 weeks of fledging.

3.2.1 Artificial diets used in petrel translocation

Over the past decade, the S/Soy diet has been routinely fed to chicks as an artificial diet during seabird translocations in New Zealand. This diet has resulted in good fledging success rates (Miskelly et al., 2009). However, it is unknown if the S/Soy diet meets the nutritional requirements of seabirds, especially during a critical period of growth. The same homogenous diet has been widely fed to various petrel species irrespective of their diverse foraging strategy.

3.2.2 Procellariiformes nutrient requirements

The nutritional requirement of burrow nesting petrels is virtually unknown (Klasing, 1998; Speer, 2015). There has only been one study on the nutritional composition of GFP chick proventricular samples, and it found that younger GFP chick diets were significantly different to older chicks (Hendriks et al., 2000). Lower fledging rates have been reported in transfers that have involved hand-raising chicks for more extended periods (Gummer *et al.,* 2014). Research into wild seabird diets often identify what prey items the diet is composed of; however, the nutritional requirements of a wild seabird diet are not necessarily the same as the foraged diet. A nutrient can be considered deficient in the diet when the addition of more of the nutrient shows an improvement in growth, reproduction, or general fitness or if a pathological condition is relieved (Klasing, 1998). The foraged diet consists of what is available to the seabird species, and the composition can be altered by seasonal variation, prey availability, foraging technique and individual preference, and may not represent the optimal or complete diet for this species. Many factors make it difficult to ascertain the nutrient requirements of wild seabirds: feeding trials are prolonged, expensive, and often require depriving the growing birds of specific nutrients to determine the importance of each element in the diet, with invasive endpoints (Speer, 2015). These studies would conflict with conservation goals in critically rare seabirds, present welfare concerns, and be limited by the financial constraints of conservation projects. In lieu of these studies, dietary requirements cannot be reliably established and instead are estimated from the wild-type diet of the species in question. Other factors to consider when formulating translocation diets are the gastrointestinal anatomy and gustatory capacity of that species; any nutritional data generated in the same or related species; and known pathologies that develop with a nutritional basis (Speer, 2015). The result is a benchmark of nutritional assumptions, that will always benefit from refinement as more is learnt about each species and through dietary trials.

3.2.3 The limitations of wild diet sampling methods.

Many methods have been used to assess the diet of wild seabirds. Previous studies have been based on behavioural observations, examining stomach contents via proventricular flushing or regurgitation in live birds; or from post mortem examinations of prey remains and regurgitation pellets (Barrett et al., 2007 as cited by Käkelä et al., 2009). Direct assessment of prey items in stomach contents is subject to unavoidable bias, as examining prey items is limited to a snap-shot in time, and discovery is subject to differential rates of digestion (Connan et al., 2005). Indirect methods have been developed to ascertain dietary input over more extended time periods, including stable isotopic signature of tissue protein and the use of lipids, particularly fatty acids and fatty alcohols, as dietary tracers (e.g., isotopes: (Cherel et al., 2005; Hobson et al., 1994) lipids: (Connan et al., 2005; Dahl et al., 2003; Horgan & Barrett, 1985; Raclot et al., 1998), and fatty acid signatures in plasma (Käkelä et al., 2009)). These studies outline the diversity and potential ratios of prey items present in the diet, giving an outline of the diet's composition. However, these methodologies are not specific enough to determine what components and proportions of the diet are essential for the growth and development of young chicks. Feeding trials assessing growth rate and energetics of different diets in seabirds are a valuable addition; however, studies of species-specific dietary traits in endangered seabirds are rare, especially in New Zealand petrel species.

3.2.4 Importance of dietary lipids

Procellariiformes (albatrosses, shearwaters, and other petrels) are unique among birds in storing large quantities of oil in their proventriculus (Warham et al., 1976). Proventricular oil is a concentrated high energy meal that occupies a relatively small volume and serves as a significant energy source to chicks (Place et al., 1989; Warham et al., 1976). The lower weight of this energy-dense oil also reduces the transport cost to the adult birds during long foraging trips (Warham, 1977). In some species, parent birds can feed proventricular oil with an energy density that is concentrated up to 35 times more the original whole prey consumed (Warham *et al*., 1976). For example, the free lipids fed to Wilsons storm petrel chicks *(Oceanites* *oceanicus*) can account for 60% of the energy delivered to chicks, while the lipids themselves accounted for only 24% of the total mass (Obst & Nagy, 1993). This concentration of energy nearly triples that of whole prey items (Obst & Nagy, 1993).

Unlike many other bird species, Procellariiformes store large quantities of fat during the nesting period (Warham, 1977). Before fledging, much of the fat is mobilised, so chicks are light enough to fly (Obst & Nagy, 1993). Proventricular oil has several benefits near fledging for translocated chicks: it is a higher energy density than body adipose stores with no energetic costs to remobilise; the oils can also be quickly jettisoned for both weight reduction or as an anti-predator defence; is a valuable energy reserve; and it can serve to store water during their first week foraging at sea (Place et al., 1991). The volume of oil needed in a diet can be hard to predict. In Leach's storm petrel *(Oceanodroma leucorhoa),* volumes of proventricular oil were found to be highly variable in all age groups of chicks and volumes of oil in the diet increased even during periods of pre-fledging weight loss (Place et al., 1991).

Across all avian species, dietary essential fatty acids and lipids are required for energy production, cell membrane synthesis, hormone production, and intracellular signalling molecules. However, little data exists concerning the fatty acid requirements of wild birds (Speer, 2015). Birds that have evolved to eat marine-based organisms may have an obligatory requirement for very long-chain omega 3 fatty acids (i.e. DHA, DPA and EPA) which are naturally high in their diets (Speer, 2015). Many carnivorous mammals and fish have lost the ability to synthesise (ARAC) and very long-chain PUFAs, as their natural diet is a rich source of these fatty acids. A nutritionally appropriate diet would also include adequate levels of ARAC, DHA and EPA as it should not be assumed that all avian species can synthesise all the PUFA that are metabolically required (Klasing, 1998).

3.2.5 Foraging strategies of seabirds sampled.

The seabird species sampled were chosen to represent diverse foraging strategies and conservation status. GFP were selected as they represent a common species with a long foraging strategy. Adult birds can forage at sea for long periods, so the chicks will often go for several days between meals (Gummer *et al.,* 2014). It is believed that their ability to withstand long periods of anorexia and dehydration is partially due to the large quantity of proventricular oil in the diet. In contrast, FS have a short foraging strategy, feeding more frequently and travelling shorter distances to forage at sea. The CP represents a critically endangered species in a niche environment that may have a uniquely different diet based on its restricted nesting habitat, as it is only found to nest on Rangatira (South East Is.) in the Chatham Islands and at two new colonies, created by translocation of chicks to Pitt Is. and the mainland Chatham Island. Both the CP and the GFP are regarded as similar species in physiology (Hendriks et al., 2000) to the critically endangered Chatham Island Petrel (*Pterodroma magenta;* tāiko*)* and therefore would be most valuable to extrapolate dietary information from.

3.3 Aims

1. This study compares three artificial translocation diets used for burrow nesting petrels in New Zealand to the wild diet of three species of Procellaiiforme seabirds, with different foraging strategies.
2. To investigate how sample collection methods can affect the analysis of wild seabird diets' nutrient composition and fatty acid profiles.

3.4 Materials and Methods

3.4.1 Wild seabird sampling

Colonies sampled

Diet samples were collected from individual chicks from 4 colonies of 3 species of burrow-nesting seabirds (Figure 30). Chicks that were selected were within 5 weeks of fledging to replicate the dietary needs of chicks selected in colony translocation. To minimise sampling stress on the chicks, all samples were collected in collaboration with other researchers or DOC staff that were already undertaking routine handling for colony monitoring or pre-transfer screening.

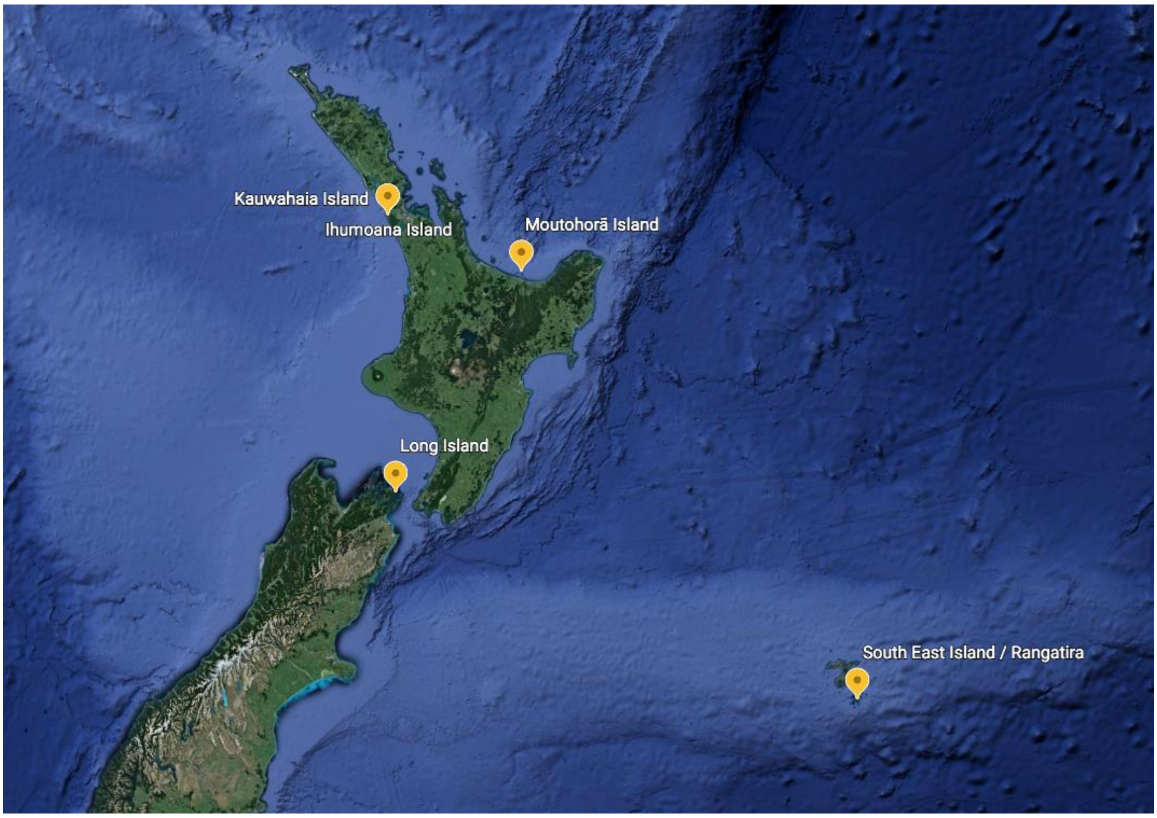


Figure 30. Map of New Zealand shows seabird colonies' locations where proventricular samples were collected in this study (Google Earth n.d.)

Colonies sampled included:

I. *Grey-faced petrels (Pterodroma macroptera gouldi), Te Henga Park, Bethells Beach, West Auckland (-36.890965, 174.439443), 26-28 November 2010. Two different sites within this single colony were sampled (Ihumoana and Kauwahaia Islands). Proventricular samples and opportunistic regurgitations were collected.*

II. *Grey-faced petrels (Pterodroma macroptera gouldi; kuia), Moutohorā Island (Whale Island), Bay of Plenty (-37.853954, 176.973685) 1-3 December 2010. Only opportunistic regurgitation samples were collected.*

III. *Chatham petrel (Pterodroma axillaris; ranguru), South East Island (Rangatira), Chatham Islands (-44.34735607956812, -176.17427839426222), 18-21 April 2011. Proventricular samples and opportunistic regurgitations were collected.*

IV. *Fluttering shearwater (Puffinus gavia; pakahā), Long Island-Kokomohua Marine Reserve, Queen Charlotte Sound (-41.108520233234294, 174.29776657366608), 7-10 January 2012. Only proventricular flush samples were collected.*

Proventricular sample collection

Wild reared chicks were sampled while their parents were away from the burrow foraging. Chicks were selected that were deemed to be near to 5 weeks from fledging, based on wing length measured from the carpal flexure to the tip of the longest primary feather using a metal ruler and weighed using a bag and calibrated spring scales. The sampling timing was inconsistent and was often determined by weather windows and coordinating with planned handling events. Chicks that were held in artificial nest boxes were easily collected directly from the box. Chicks in natural burrows were removed through the burrow entrance, either by hand in short burrows, or in the longer burrows using a stick with soft net stocking attached at the end to ensnare the chicks' beak. Chicks were then slid or gently walked out of the burrow. During handling, all the chicks were weighed and their wing length measured, the down cover was recorded as a percentage of the total and a general physical exam was given by the handler to screen for any abnormalities.

Proventricular contents were collected by holding the chick in a sitting position with its neck extended and a soft silicon feeding tube was placed into the proventriculus. The tube measured 10mm in diameter and was cut to length so that the tube was advanced just past the thoracic inlet. Isotonic saline was flushed into the chicks, with a maximum of 20 ml for CP and 60 ml for GFP and FS. The volume flushed in was reduced slightly if the proventriculus felt full or if the chick beak commissures flared, indicating it was ready to regurgitate. The tube was removed, and the chick was held with its head pointing approximately 120 degrees downward. The base of the abdomen was gently massaged to stimulate the chick to regurgitate. After regurgitation, chicks were immediately returned to their burrow. If the chick did not regurgitate after one PVF flushing event, then no further attempts to flush the chick were made and the chick was returned to the burrow.

Additional samples were opportunistically collected if birds that spontaneously regurgitated (REG) during the handling process. Sample were excluded from analysis if they became contaminated by touching the ground or if less than 80% of the regurgitation was caught.

All samples were collected directly into either a clean plastic container for larger chicks or a zip lock plastic bag. Air was removed from the plastic bags to prevent oxidisation and the samples were wrapped in tin foil to avoid vitamin degeneration due to UV light. Samples were kept chilled or frozen at -20 °C until they could be stored in a -80°C freezer unit. For sample transport that was less than 24 hours, the samples were placed in chilly bins with dry ice, for trips less than 4 days in the field a -20 C portable freezer unit was used and for samples that needed to be stored for 3 weeks then they were placed in 5ml cryovials in a liquid nitrogen dewer.

Artificial diets

The following artificial diets were used to compare the nutritional composition with the samples collected from wild seabird chicks:

a. Brunswick™tinned sardine in soya oil (S/Soy)
b. Mazuri fish eating seabird analogue diet® with Watties fish oil added (M/Fish)
c. Brunswick™ tinned sardine in spring water (S/Fish)

See Chapter 2 methods for further details, Table 1 & Table 3.

3.4.2 Laboratory analysis

All samples were sent to the Nutrition Laboratory, Institute of Food, Nutrition & Human Health, Massey University, Palmerston North (MUNL) within three weeks of collection for analysis.

Proximate analysis:

Each sample was weighed and converted to dry matter by using heat volatilisation. Each diet sample from every individual bird was analysed for moisture, fat, protein, carbohydrate, and ash content.

Laboratory proximate analysis used the following methods for each component from the Official Methods of Analysis from the Association of Official Agricultural Chemists, (AOAC): Protein: Leco, total combustion method from the (AOAC) 968.06. Fat: Soxtec extraction, AOAC 991.36. Moisture: Convection oven 105 °C, AOAC 930.15, 925.10 and Ash: Ash: Furnace 550 °C, AOAC 942.05. The Nitrogen to protein conversion ratio was 6.25.

In summary, the fat was dissolved by washing the sample with petroleum-ether by refluxing in a Soxhlet apparatus. The solubilised fat was then collected in the distillation flask, and the increase in weight of the flask was measured to represent the dissolved fat content. The samples were ignited at 500(C to burn off all organic material to measure the ash content. The inorganic material not volatilised at this temperature was recorded as the ash content.

Pooled sample analysis:

Samples with no significant difference on proximate analysis were pooled for further nutritional analysis. Samples collected from the wild seabirds' mineral, vitamin, and fat content were analysed and compared to results from a previous study of GFP by Hendriks et al. (2000).

The fatty acid compositions of the three artificial diets fed to seabirds were compared with pooled samples from PVF and REG samples by screening for 36 fatty acids. The composition data is a relative proportion of each fatty acid, where all fatty acids are summed for a given sample total of 1.

Fatty acids were analysed using methods described in Sukhija & Palmquist (1988). Amino acids were analysed using methods described in Rutherford & Moughan (1998).

In summary, fatty acids were analysed as follows. The dry sample was suspended in toluene. Fatty acids were methylated by using methanolic hydrochloride (a mixture of methanol and acetyl chloride) in culture tubes. Samples were vortexed and heated at 70°C for two hours for methylation. After methylation, samples were cooled on ice, and 5.0ml of potassium carbonate and 2.0ml of toluene were added. Samples were then vortexed and centrifuged at 2500rpm for seven minutes at room temperature to separate the solvent layer containing methyl esters and the aqueous layer. Gas chromatography was performed using a Shimadzu GC-2010 Plus Gas chromatograph equipped with a flame ionisation detector (FID), fitted with a SupelcoTM-2560 Capillary Column 100m x 0.25mm x 0.2um film thickness. The oven temperature was programmed to hold at 140°C for 5min then to increase to 240°C at the rate of 4°C /min, hold for 38min. Injector temperature was 250°C, Detector temperature 255°C. Standards were purchased from Sigma-Aldrich Co. (Sukhija & Palmquist, 1988)

Amino acids were determined on duplicate 5mg samples using a Shimadzu ion-exchange HPLC system utilising post column o-phthalaldehyde derivatisation and fluorescence detection, following hydrolysis in 6M HCl containing 0.1% phenol for 24 h at 110°C in an evacuated sealed tube. For the determination of cysteine and methionine, hydrolysis was preceded by oxidation with performic acid at 0°C in an ice bath for 16h, followed by neutralisation with hydrogen bromide (Rutherfurd & Moughan, 1998).

Mineral analysis was performed on either a Perkin Elmer Sciex Elan 6000, Elan 6100 DRC, or Elan DRCII Inductively Coupled Plasma – Mass Spectrometer (ICP-MS). The system consisted of a variable speed peristaltic pump, nebuliser, argon gas plasma (1500 W), vacuum chambers, quadrapole, and a combined pulse counting/analog detector. Each element is monitored at an isotope(s) chosen for its abundance/sensitivity and freedom from known interferences.

Vitamin analysis was run using AOAC international methods: for vitamin A, the Carr-Price modified method, AOAC 974.29 (4), for Vitamin E the AOAC official modified method 971.30 and for Vitamin D3 the AOAC official method 982.29 (modified). In summary, for analysis of Vitamin A and E a known weight of oil or sample was saponified with ethanolic potassium hydroxide solution and the vitamins A and E were extracted with n-hexane:ethyl acetate (80:20). The hexane was gently evaporated under the rotary evaporator (36-38°C) and the remaining residue was dissolved in HPLC methanol. The vitamins were then quantified using reversed phase high-performance liquid chromatography. For Vitamin D3 a known weight of oil or sample is saponified with ethanolic potassium hydroxide solution and vitamin D is extracted with n-hexane:ethyl acetate (80:20). The hexane is gently evaporated under the rotor vapour (36-38 ºC) and the remaining residue is passed through a C18 (EC) SPE cartridge for a cleanup to separate from interfering substances. The Vitamin D3 (cholecalciferol) is quantified using the Reverse-phase HPLC.

Differences in significant fatty acids of particularly interest to this study were described and compared between different translocation diets and between species. Fatty acids compared included: DHA, EPA, NERV, DPA, ARAC, LIN, ALIN, PAL and OLE.

3.4.3 Statistical analysis

All sample results were screened for normality with probability plots or Andison darling normality tests; data was transformed as needed with log base 10, natural log or Johnson transformations. All components of the proximate analysis, wing length and weight were compared between sampling method groups using either paired or two sampled t-tests, one way ANOVA’s or Mann-Whitney tests (for non-parametric data). Comparisons of GFP sampling methods included comparisons within individuals, between individuals and between colony sampling locations. Three chicks were sampled by both methods and of these only REG samples were included in further comparative analysis between sampling groups.

Proximate analysis results of fat, protein and ash percentage from GFP PVF samples (group 1) and GFP REG samples (group 2) (see Table 16) were compared with the mean of pooled proximate analysis results from Hendriks et al. (2000), using one-sample t-tests. The REG samples fat percentage could not be transformed to a normal distribution, so a one-sample sign test was used to compare the medians of fat percentage to the Hendricks study average.

Box plot graphs compared differences between species and then outliers were removed. Samples were compared by general linear model (GLM), with sampling method nested within species and Tukey pairwise comparison with 95% intervals. The fat percentage by species was normally distributed, so a GLM was run comparing species, however, the GFP REG samples could not be transformed to a normal distribution. Therefore a Moods median test was used to compare sampling methods between all species. The test was repeated, excluding FS, to compare only CP and GFP sampling methods. Minitab statistical software programme was used for all statistical analysis.

Proximate analysis results for GFP and FS that were normally distributed were pooled for analysis, and CP samples were pooled irrespective of normality results as the sample volume was very small and needed in entirety for further nutritional analysis.

Principal component analysis (PCA) graphs were used to identify significant sources of variation and were analysed using the R statistics programme. Four fatty acids were not detected in any sample, and so were excluded from the analysis (Heptadecenoic C17:1n7 - cis-10, Linolelaidic C18:2n6t, Heneicosanoic C21:0). The data was transformed by square root to give rarer fatty acids a greater weighting, as the interaction between all fatty acid's quantity and seabird health is not a linear relationship.

Differences in significant fatty acids were described and contrasted in PCA and bar graphs. Fatty acids compared included: DHA, EPA, NERV, DPA, ARAC, LIN, ALIN, PAL and OLE.

3.5 Results

3.5.1 GFP sampling method and proximate analysis.

GFP frequently regurgitated during handling, while FS and CP chicks were far less likely to spontaneously regurgitate.

GFP diet collection

In total, samples were collected opportunistically from 53 birds that were being handled for monitoring processes. Samples were excluded from analysis if PVF did not produce a sample, if a sample became contaminated or if all of the REG was not caught. Proximate analysis tests were run on 31 of 53 samples, from 28 chicks. Samples consisted of 11 PVF and 20 REG, (Table 14). Three chicks that regurgitated were then sampled by PVF to compare the effects of the sampling methods (REG and PVF) in the same bird.

The samples all showed a heterogenous consistency with varying levels of oil. A variety of prey items were noted in the regurgitant, including white fish flesh, fish spines, red krill, tentacles, otoliths, squid eyeballs and squid beaks.

Table 14. Grey-faced petrel proventricular samples were collected at three locations using two different sampling methods.

Sampling method.	Sampling location			Total
	Ihumoana Is	Kauwahaia Is	Moutohorā Is	
Proventricular flushes	11*	0	0	11
Regurgitations	5*	5	10	20
Total	15	5	10	

**3 chicks were sampled by both spontaneous regurgitation and proventricular flushing.*

Comparing sampling methods from the same chicks

There was no evidence of a difference between sampling methods used on the same chick for proximate analysis results of fat, protein, ash and residual dry matter percentage (paired t-test fat%: t=-2.2 p=0.16: protein% t=1.27 p=0.33, ash % t =2.52, p=0.19 and dry matter percentage residual t=-1.11 p=0.38). The REG samples showed significantly lower moisture percentage and higher total dry matter percentage (t=-6.21, p=0.025) (Table 15) than the samples obtained by PVF on the same chick.

Table 15. Summary of the effect sampling method and location have on proximate analysis results in two species of petrel chicks and compared to a previous study of regurgitation samples from grey-faced petrel chicks (Hendriks et al., 2000).

Species	Comparison of sampling methods	Total DM %	Residual DM%	Moisture %	Protein %	Fat %	Ash%
Grey-faced petrels	*REG vs PVF within the same chick*	↑	–	↓	–	–	–
	REG samples within the same colony	–	–	–	–	–	–
	*REG samples between distant colonies**	–	–	–	–	–	–
	REG vs PVF in the same colony	↑	–	↓	–	–	↓
	REG vs PVF between colonies	↑	↑	↓	↓	↑	↓
	REG compared to Hendriks previous results	x	x	X	↑	↓	↓^
	PVF compared to Hendriks previous results	x	x	X	–	–	↓^
Chatham petrels	*REG vs PVF within the same colony*	–	x	X	–	–	–

↑↓ results indicate significant increases or decreases in REG samples p=< 0.05

– = no significant difference

x = not reported

↓^= significantly lower result compared to results from (Hendriks et al., 2000) Grey-faced petrel pooled regurgitation samples collected from the Te Henga colony.

Samples were collected by either proventricular flushing (PVF) or REG (REG)

** A significant difference in wing lengths between regurgitation sample groups was found.*

Regurgitation samples compared by location.

There was no evidence for an effect of sampling location on REG sample proximate analysis results, when compared within the same Te Henga colony (Ihumoana Is n=5 and Kauwahaia n=5) (2-sample t-tests: DM% total t=-0.78, P=0.476, DM% residual t=-1.62, p=0.149, ash% t=-0.89, p=0.422, moisture% t=0.78, p=0.476, fat% t= -0.78, p=0.464 and protein t=1.11, p=0.303), or when compared with the geographically distinct colony Moutohourã Island, 250km away, (n=10), (one-way Anova: Total DM % f=0.5, p=0.615, Total DM% Residual f=2.59, p=0.104, ash % F = 0.86, p=0.442, moisture % f= 0.5, p=0.615, fat % f=1.25, p=0.311, protein % f=1.71 p=0.21), (Table 15).

A similarity in regurgitation samples between all three locations was observed despite birds having a significant difference in wing lengths (as a proxy for chick age), with Moutohourã Island chicks having slightly smaller wing lengths (one-way ANOVA f=3.64, df=2,17, p=0.048), ranging from 162-237mm, (mean = 199mm, s.d= 27.33), compared to the slightly older or larger chicks on Ihumoana (mean=248mm, s.d=22.19) and Kauwahaia (mean=228.2mm, s.d=53.2), ranging from 171-279mm, (Figure 31). No difference in mean weight was observed between these two groups of birds (Te Henga mean = 641.1g, Moutohourã Island mean = 572g; 2 sample t-test t=-1.17, p=0.272). For further analysis, all the GFP REG samples were pooled, and this represents chicks spanning wing lengths of 162-279mm, averaging 235.5mm and weights 360-940g, averaging 604.7g.

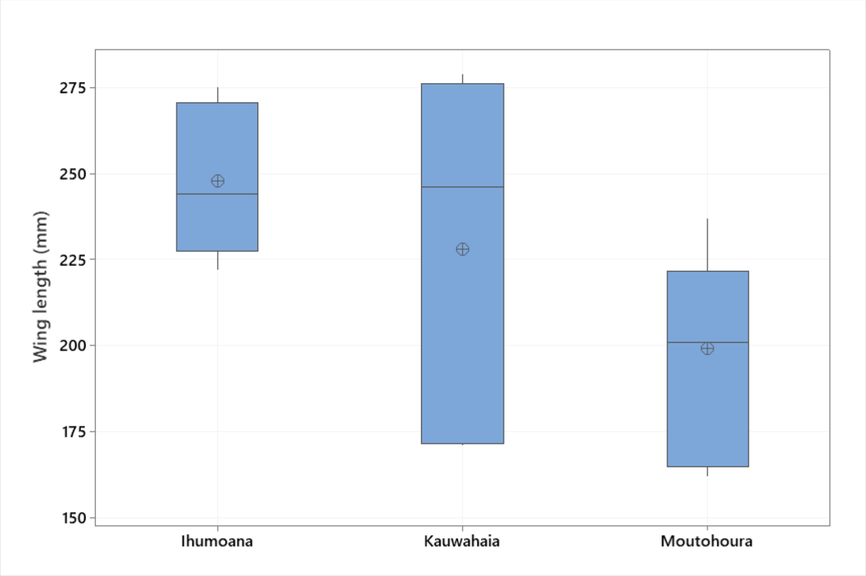


Figure 31. Boxplot of grey-faced petrel chick wing lengths within the regurgitation sample groups, showing a significant difference between the Te Henga colony (Ihumoana and Kauwahaia) and the Moutohourã Island colony.

The effect of sampling method on GFP chick proximate analysis

The sampling method had a significant effect on GFP proximate analysis results both within and between colony sites. Within the Te Henga colony comparisons of n=8 PVF and n=10 REG samples of proximate analysis showed PVF samples contained significantly lower total dry matter percentage, with higher ash and moisture percentages compared to REG samples, (t=-3.39, p=0.004, t=2.18, p=0.047, w=108, p=0.005, respectively, 2 sample t-tests and Mann Whitney comparison). Residual dry matter, fat and protein percentages were found in similar proportions (t=-1.62, p=0.125, t= -1.34, p=0.2 and t=1.12, p=0.279), (Figure 32, Table 15). There was strong evidence that the sampling method affected all aspects of the proximate analysis in GFP, when the sample size was expanded to include comparisons between all colony locations (Figure 32, Table 15). Proximate analysis results were compared in 2 groups; Group 1 (PVF samples from Ihumoana n=8) with Group 2: (n=20 regurgitation samples consisting of n=5 Ihumoana, n=5 Kauwahaia and n=10 Moutohorā Is) (Table 16). PVF sampling produced significantly higher proportions of ash, protein and moisture (t=3.1, p=0.007, t=2.29, p=0.035 t=4.88, p=<0.000 respectively) while collecting samples by REG resulted in significantly higher amounts of fat and with higher total and residual dry matter percentage (w=71, p=0.024, t=-4.88, p=<0.000, t=-2.54, p=0.026, respectively), (Figure 32, Table 15). Given the significant differences from sampling methods, the GFP samples were pooled into two separate groups based on sampling method (group 1 and group 2) and full nutritional analysis was run on each group independently, Table 16.

Table 16. Pooled groups of diet samples for nutritional analysis, comparing three species of petrel.

Sampling method	Grey-faced petrels		Chatham petrel	Fluttering shearwater
	Group 1 n=8	Group 2 n=20	n=13	n=10
Proventricular flushing	8	0	5	10
Regurgitation	0	20	8	0

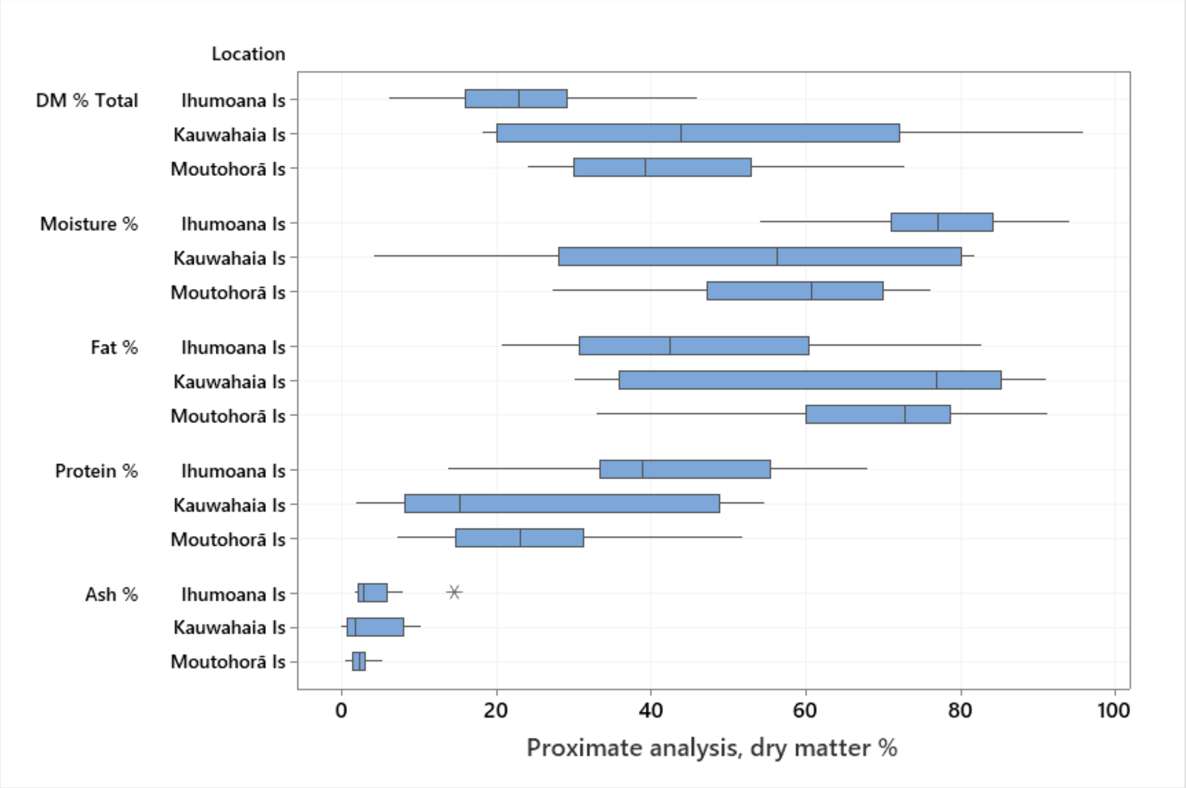


Figure 32. Boxplot of grey-faced petrel chick wild diet proximate analysis within colony sites Te Henga (Ihumoana & Kauwahaia) with a distant colony, Moutohorā Is near Whakatane.

Boxplots show the median, interquartile range, range, and outliers (*) for each group.

The Ihumoana Is samples contain only proventricular flushes (n=8), while Kauwahaia Is and Moutohorā Is include only regurgitation samples (n=5 and n=10, respectively).

Chick morphometric comparisons between pooled proventricular flush and regurgitation sample groups.

Weight and wing length were similar between the pooled GFP groups 1 and 2 irrespective of sampling method (t=-0.05, p=0.965 and t=-0.26, p=0.798 respectively) (Figure 33). Within both the pooled GFP sample groups weight ranged from 360-940g (av=614.6g s.d=116.1g) and wing lengths spanned 162-310mm (av=222.5mm, s.d=42.6mm) (Figure 33). This age and size range encompasses the recommendations for GFP at the time of selection for transfer (Gummer, Taylor, Collen, et al., 2014)

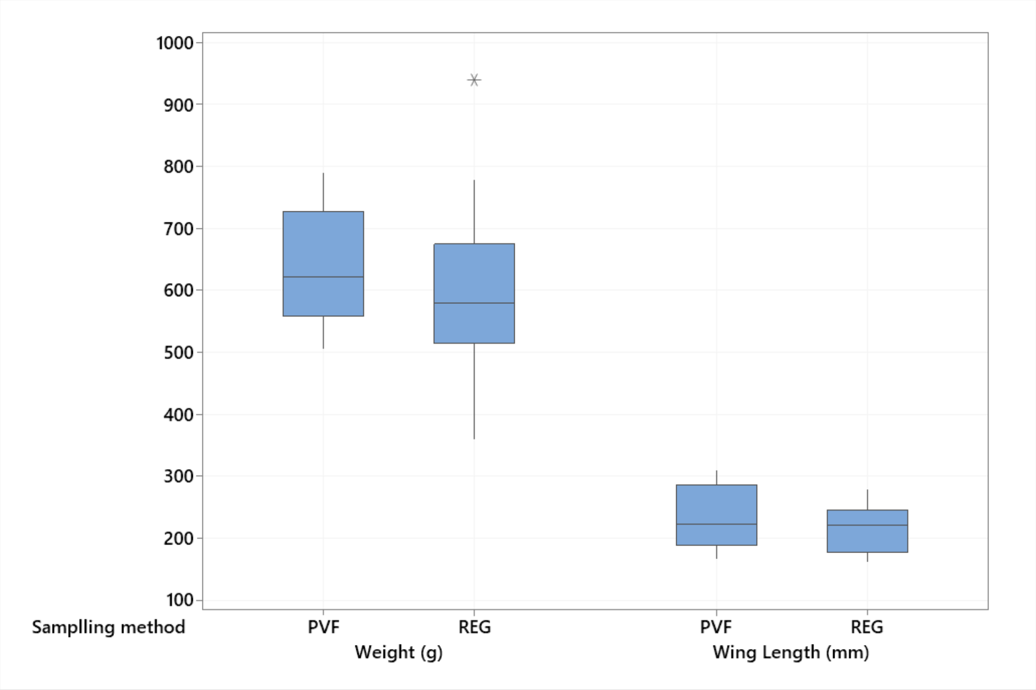


Figure 33. Boxplot showing the relative size of grey-faced petrel chicks (measured by weight and wing length) included in pooled proventricular flush (PVF) group 1 and pooled regurgitation (REG) group 2 samples.

Comparison of GFP proximate analysis results to previous studies

PVF proximate analysis results were more similar to the previous findings in Hendriks' et al. (2000)'s study than REG samples were, in spite of the fact Hendricks' study collected REG samples. PVF showed no evidence of difference in fat or protein %, (t=0.56, p=0.59, t= -0.65, p= 0.54), while REG samples had significantly higher fat and lower protein %, (fat% p=0.012, protein% t= -4.27, p= <0.001). Ash was notably higher in Hendriks' et al (2000) study compared to both PVF and REG samples (t=-5.25, p=0.001, t=-20.79, p= <0.001) (Figure 34).

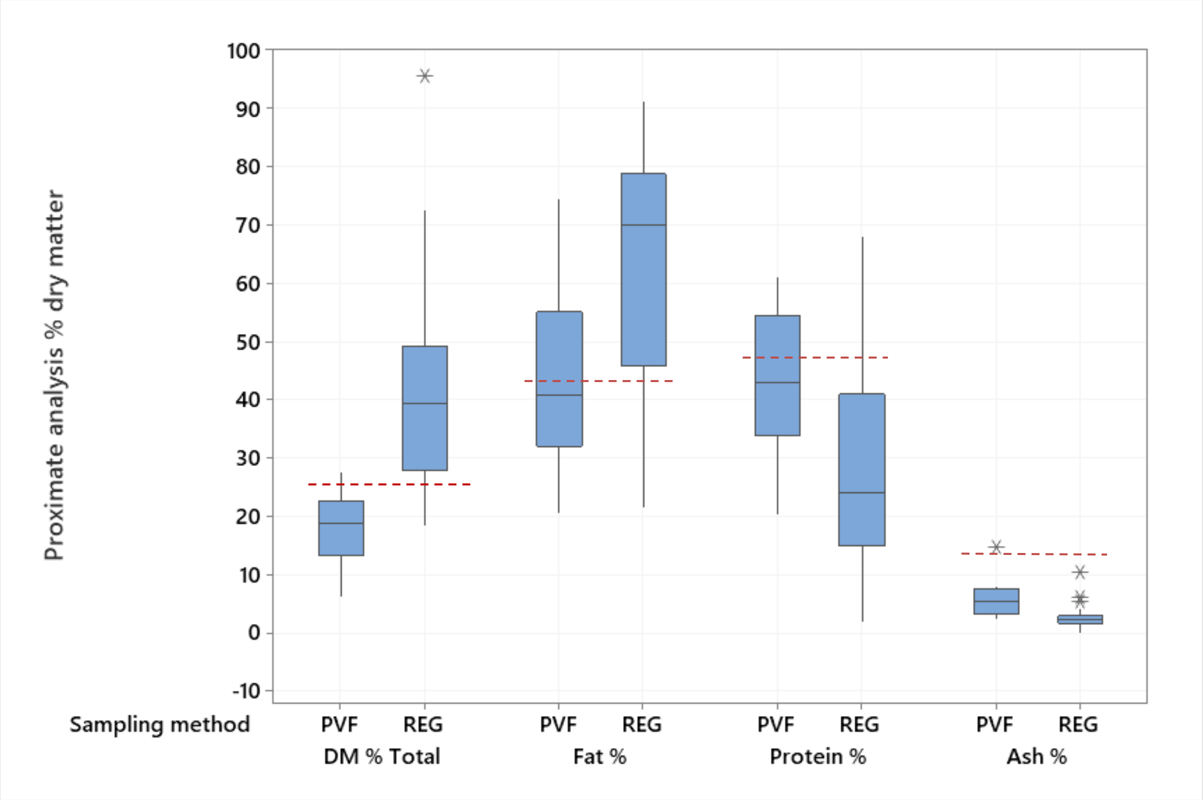


Figure 34. Components of the proximate analysis of grey-faced petrel diet samples, comparing sampling methods proventricular flushing (PVF) (n=8) with spontaneous regurgitation (REG), (n=20).

> *The red dashed line represents the only previously published proximate analysis results of grey-faced petrel chicks, from n=43 pooled REG samples collected from the same colony in 1997 (reported in g/100gDM) (Hendriks et al., 2000).*

3.5.2 CP sampling effect and proximate analysis

CP samples included 13 proximate analysis samples with 5 proventricular flushes and 8 regurgitations. The PVF sampling method collected 5 samples from 10 CP chicks, while eight REG were collected opportunistically. Samples contained remnants of squid beaks, eyeballs, fish spines and krill exoskeletons.

PVF sample sizes collected after a 20ml flush ranged from 16ml to 36ml with an average of 21ml, whilst REG samples ranged from 2-24 ml with an average of 12.8ml. However, 5 of the 8 REG samples collected missed catching a small proportion of oily regurgitation when the chicks were first picked up. During the freeze-drying process, MUNL reported that four particularly oily samples (3 PVF and 1 REG) leaked from the tube due to the high pressure from the oils. Unfortunately, the weights after freeze-drying will be inaccurate in these four samples. Ideally, only complete samples would be compared and pooled; however, due to the limited size of samples after freeze-drying, all 13 samples collected needed to be pooled, irrespective of sampling method, so there would be enough dry matter available to enable further nutritional analysis. Within the pooled CP sample, 3 of the 5 PVF samples and 5 of the 8 REG samples will not fully represent the sampling method and are likely to slightly underestimate the proportion of oil in the diet.

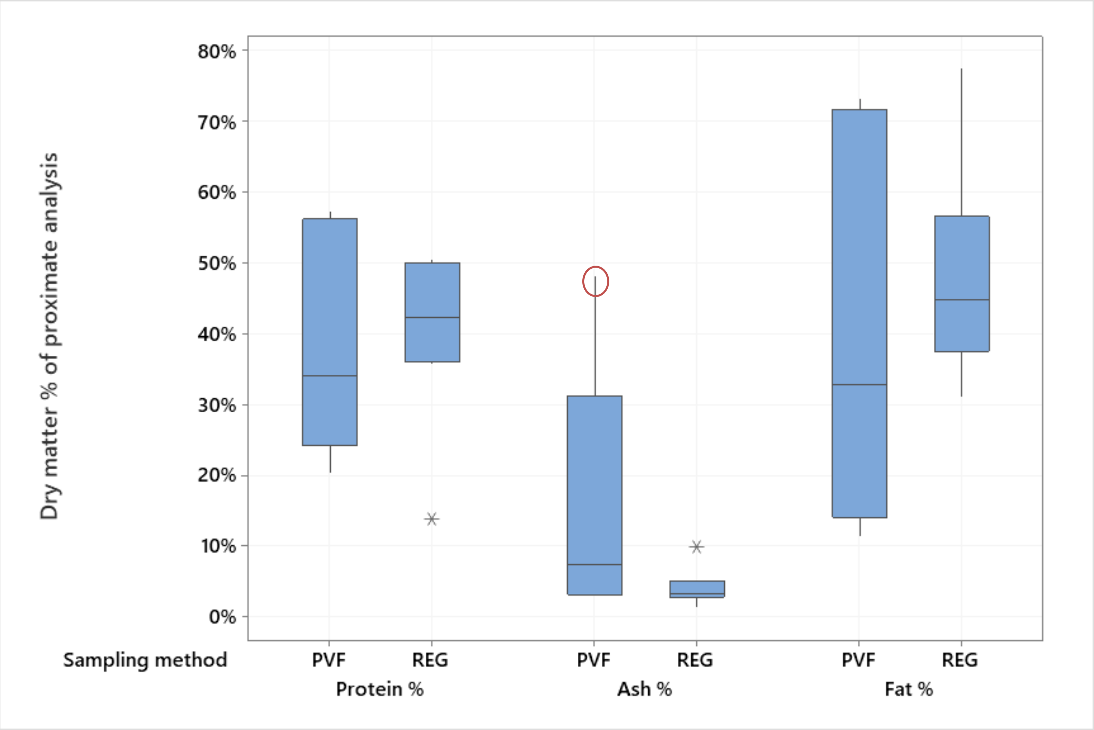


Figure 35. Chatham petrel proximate analysis results comparing samples collected by proventricular flushing (PVF) and spontaneous regurgitation (REG).

A boxplot shows each group's median, interquartile range, range, and outliers (*).

○ *The red circle represents the Ash sample SE3.*

Comparison of all 13 CP samples found no effect of sampling method on proximate analysis results before pooling, (DM summary % t=0.85 p=0.424, protein % t=-0.14, p=0.90, ash% t=1.6 p=0.170, fat % t=-0.52, p=0.628), (Table 15).

Weight and wing length were similar between the PVF and REG groups, (t=-0.92, p=0.394, and t=-1.49, p= 0.166 respectively). The weight of chicks sampled ranged from 265-355g, (av= 310g, s.d= 26.5) while the wing length ranged from 99-193 mm (av= 138.8mm, s.d = 30.46).

A notable outlier was SE3, a sample from a chick with an ash content of 48% (34% higher than any other sample) (Figure 35, Figure 38). In this chick, 15mls of the sample was recovered from a 20 ml flush and included krill exoskeletons and squid beaks, similar to other samples. The entire regurgitation was collected; however, the actual vomit appeared to be less solid and well digested than the other samples. To investigate the cause of high ash percentage in several samples, a proximate analysis was run on the gizzard contents with a high volume of squid beaks from a necropsied chick that had died from reasons unrelated to this study (Figure 36). The protein dry matter percentage was 70%, nearly double that of any other samples; however, there was an insufficient sample to determine the proportions of fat or ash.

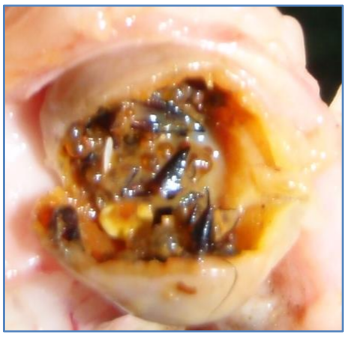


Figure 36. Gizzard content packed with squid beaks from a necropsied Chatham petrel chick.

3.5.3 FS sampling effect and proximate analysis

Of the 10 FS chicks sampled, all 10 produced PVF samples, with 10 to 40 ml of regurgitant returned after a 60ml proventricular flush, averaging 21 ml a sample. Based on the disturbance of sticks placed at the entrance of burrows (used to signify when chicks are fed by parents or chicks emerge from entrances) and watching adults frequent the burrows, it is estimated that nine samples were collected within two hours to 24 hours of the chicks recently being fed, while one sample that may have been fed within the last 24-48 hours. This older sample had more oil present, and no firm matter present, with the lowest protein content of 21.2% and the highest fat content of 54.6% (Figure 37). In comparison, samples from chicks that were suspected to be recently fed contained chunks of white fish meat and fragments of krill.

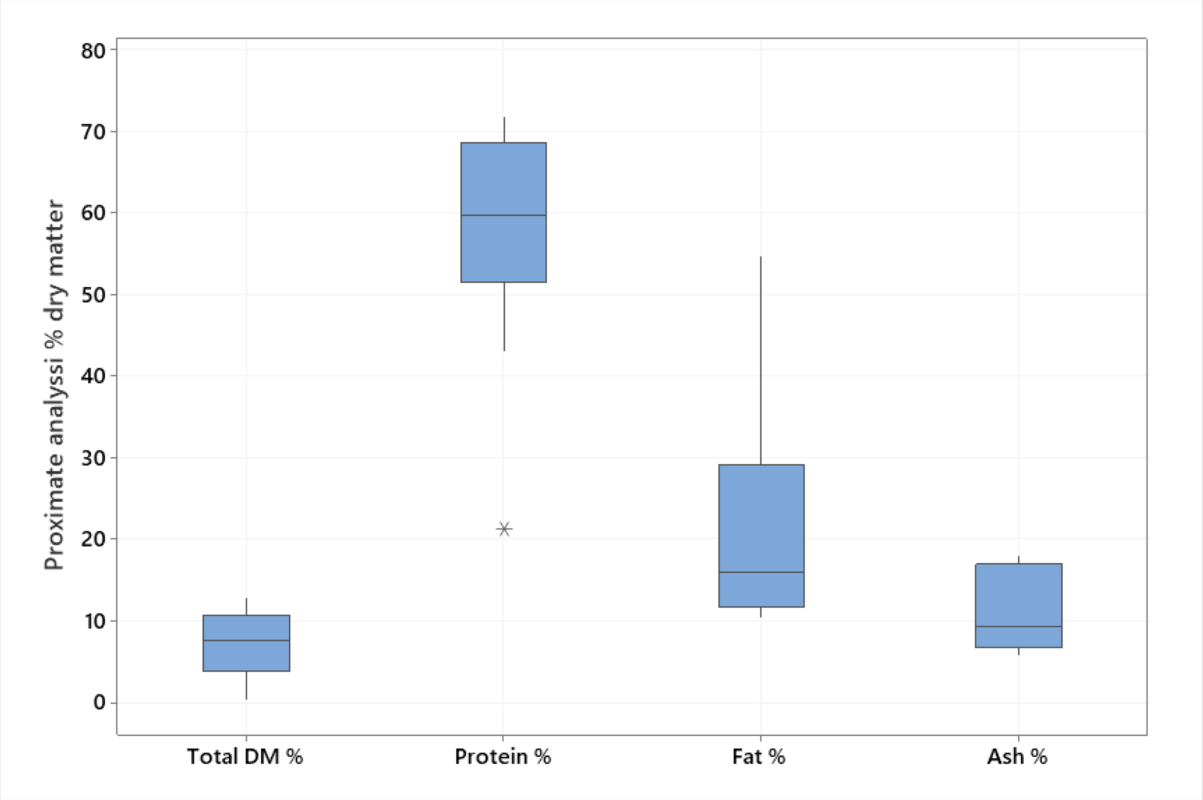


Figure 37. Boxplots showing the proximate analysis of diet samples from fluttering shearwater chicks (n=10) collected by proventricular flushing.

> *The notable outlier with low crude protein and high fat is from a chick suspected to be fed between 24-48 hours previously, rather than the remainder suspected to be fed in the last 24 hours.*

3.5.4 Comparison of proximate analysis content between the three species of petrel

The proximate analysis results were notably different when compared between species, and the effect of the sampling method contributed to this variation. Figure 38 shows the differences in the main components of the proximate analysis. Two outliers were removed, including the CP sample with an ash percentage of 48% and the FS sample with a protein percentage of 21 %, Figure 38. Both outlier samples were highly likely to have been digested for longer periods than other samples in the group.

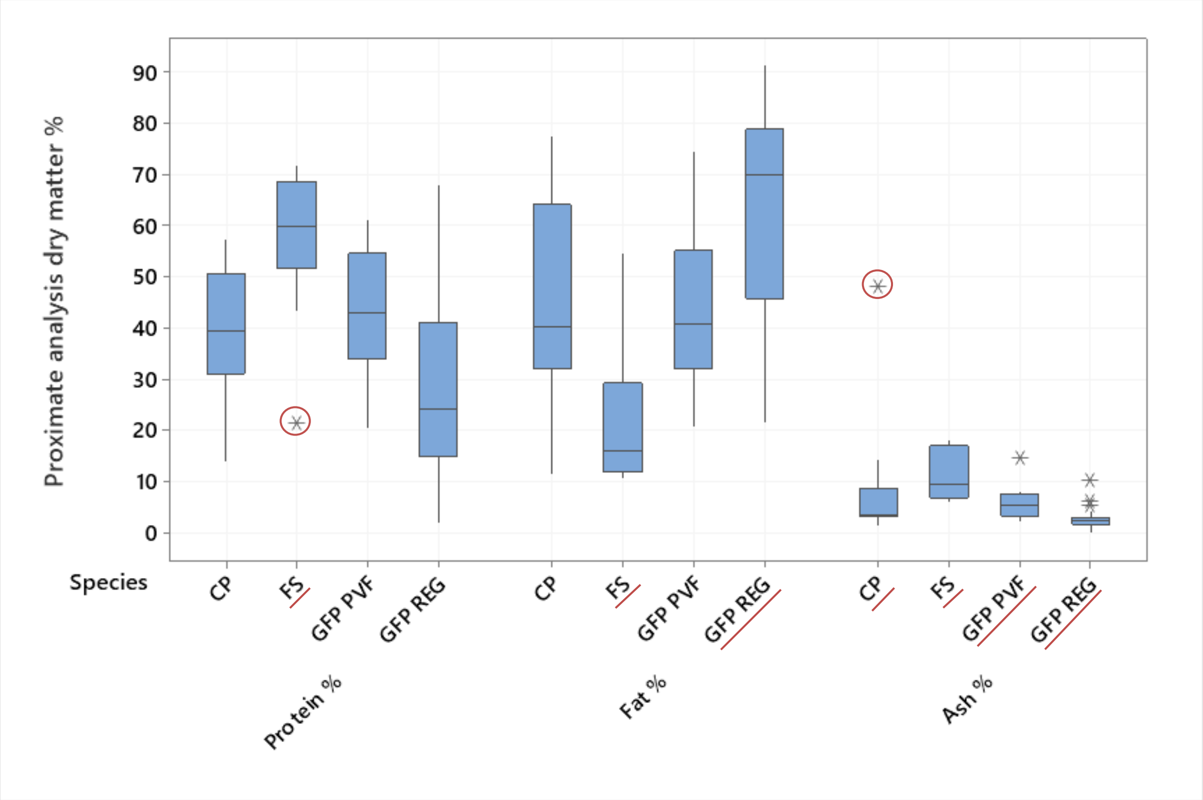


Figure 38. Comparison of pooled proximate analysis results across three different species of petrel (Chatham petrels (CP), fluttering shearwaters (FS) and grey-faced petrels (GFP). GFP samples are grouped into proventricular flushes (PVF) or regurgitation (REG) samples, CP is a pool of both sampling methods and FS were collected by PVF.

**The stars in the FS protein % and CP ash % groups are the 2 outliers that have been removed for further statistical analysis*

— *Red underlined results are statistically different p=<0.05 to the rest of the group.*

○ *Red circles represent the two outliers removed from statistical analysis.*

The proportion of protein was significantly higher in FS chicks (mean = 61.19 %; F=9.33, df = 2,44, p<0.001), while the proportions of protein in CP and GFP samples were similar (Tukey T value=-0.8, p=0.704, mean CP 40.09% and GFP 32.51%) irrespective of sampling method which showed only weak evidence for an effect between species (F=2.49, p=0.095). The relationship between variables is likely to be more complex as this GLM model only predicted 26% of the variance in the data set (R squared = 39.95%, R sq (adj for ties) = 34.5% and R-squared predicted =26%) (Figure 38).

The proportion of fat was significantly lower and less variable in the daily feeding FS species (F=14.47, df = 2,46 p<0.001) compared to long strategy foragers, CP and GFP, which both showed higher and more variable proportions of fat in the diet (Tukey T=1.49, p=0.306),

(Figure 39). However, very little of the data variance was captured in this GLM model (R squared=38.62%, R sq (adj)=35.95% and R pred =32%). GFP REG samples had exceptionally high and variable proportions of fat that could not be normalised and this was a source of significant difference at all stages of analysis. Fat levels were compared by sampling methods between all three species (Chi -squared 11.5, p=0.009), sampling method irrespective of species (χ^2=9.32, p=0.002) and between only CP and GFP sampling methods (χ^2= 4.29, p=0.038) using Moods median tests (Figure 39). Interestingly, in contrast to GFP, the average proportion of fat was higher in CP PVF samples than in CP REG samples. However, this CP data is limited by very small sample sizes and loss during analysis (Figure 39).

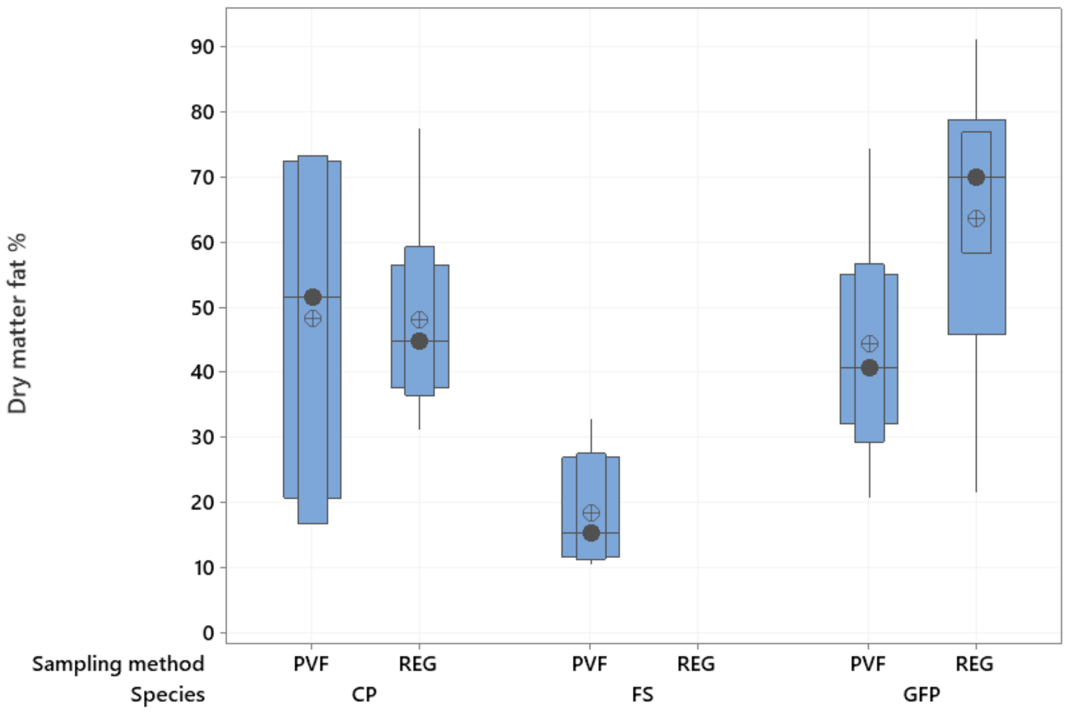
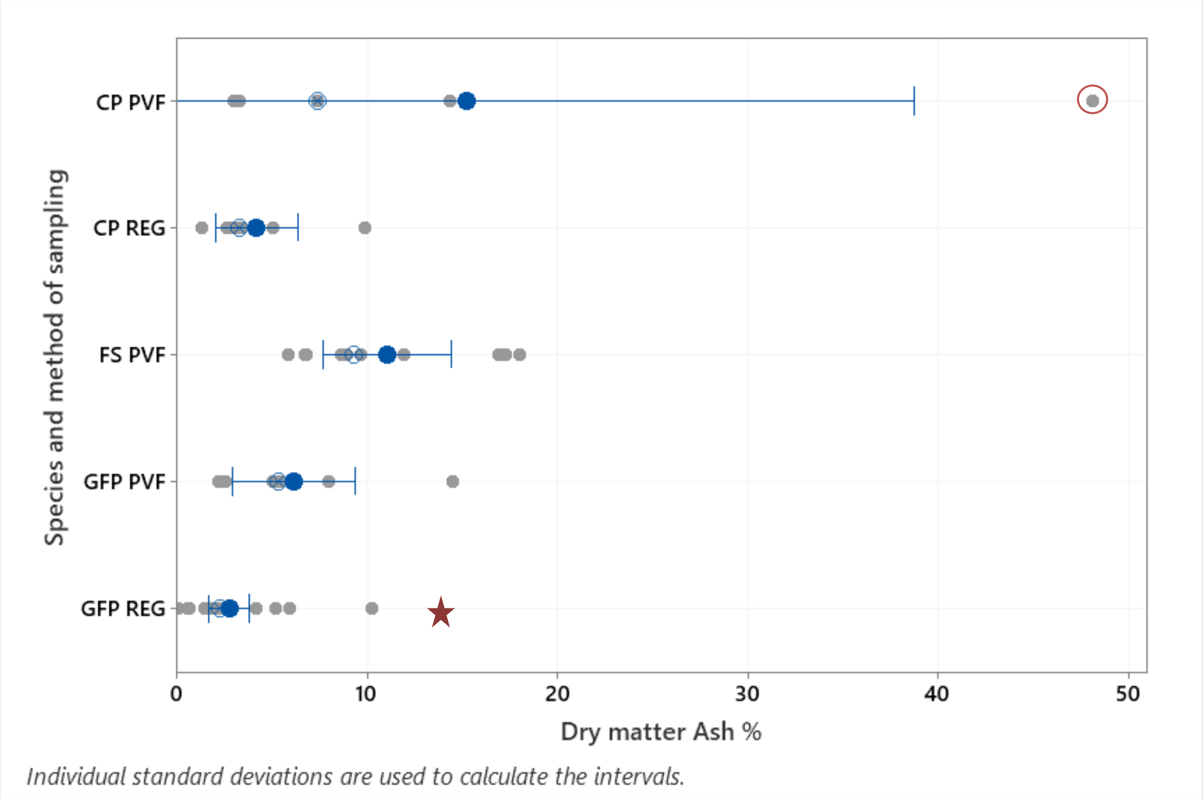


Figure 39. Boxplot showing the difference in proportions of fat in proximate analysis results of Chatham Petrel (CP), fluttering shearwater (FS) and grey-faced petrel (GFP)) comparing two sampling methods, proventricular flushing (PVF) and spontaneous regurgitation (REG).

This data set has two outliers removed so each group, therefore CP PVF n=4, CP REG n=8, FS n=9 GFP PVF n=8 and GFP REG n=20. The solid dot represents the median, and the unfilled dot represents the mean. The inner box represents the median 95% confidence interval, and the outer box represents the median 25% and 75% quartile.

The proportions of ash in all chicks showed considerable variation both between species and sampling methods, (F=8.75, df= 2,43, p=0.001 and F=5.68, df= 2,43 p=0.006 respectively). The variability in the sampling method is well illustrated by one chick that was sampled using both methods. The ash content increased from 2.9% in REG samples to 18.9% in PVF samples. Outliers with higher ash values were present in each species and sampling method (Figure 40). There are likely other sources of variation as species and sampling method only accounted for 32% of the predicted variability. (R squared=45.78%, R sq (adj) =40.74% and R sq (pred)=32.30%). FS, on average, had a mean ash content that was markedly higher than CP and GFP chicks (T=2.7 p=0.029 and (t=-1.15, p=0.000). However, no REG samples were collected, and this may account for some of the differences. Samples collected by PVF had a significantly higher ash content than REG samples, irrespective of species (T=3.28—5.17 and p=0.000-0.024), with the one exception of CP chicks where REG and PVF samples were similar (once outlier of 43% was removed).

The proportion of ash as a substantial component of the proximate analysis in Hendriks *et al.* (Hendriks et al., 2000) the initial GFP study (14g/100g DM= 14% DM) and was a repeatable finding in this study, in PVF samples (av 6.11, range 2.1-14.5 %DM) but not in regurgitation samples (av 2.77 range 0-10.2% DM), (Figure 40).



Individual standard deviations are used to calculate the intervals.

Figure 40. Proximate analysis ash content displayed in 95% confidence interval plot, comparing Chatham Petrel (CP), fluttering shearwater (FS) and grey-faced petrel (GFP) samples collected either by proventricular flush (PVF) or regurgitation (REG).

● *Solid blue dot represents the mean, the light blue cross represents the median.*

○ *The red circle represents the CP outlier of 48% ASH which is included in this graph but not the comparative statistical analysis.*

★ *The red star represents the mean proportion of ash reported by (Hendriks et al., 2000) when regurgitation samples from 43 GFP chicks were collected from the Te Henga colony in 1997.*

Minerals in grey-faced petrels pooled samples compared to a previous study and between species

The mineral analysis results of the GFP diet in my study compared to the previous study by Hendricks et al. (2000)shows that manganese, zinc, selenium and the calcium: phosphorus ratio are all markedly lower. Higher sodium was present in samples collected by proventricular flushing, compared to both the Hendriks *et al.* (2000) study and my samples collected by regurgitation (Table 17). There was no evidence of a difference between the vitamin A and E concentrations between the two sampling methods I used. Vitamin D3 was below the limit of detection of the assay. Hendriks *et al*. (2000) did not perform vitamin analysis on their samples.

Table 17. Comparison of grey-faced petrel mineral and vitamin content in pooled chick proventricular samples, comparing two different sampling methods with the only previously published study undertaken by Hendriks *et al* (2000).

Pooled analysis	GFP PVF Group 1 n=8	GFP REG Group 2 n=20	GFP REG Hendricks *et al* 2000 n=43	GFP PVF Group 1 n=8	GFP REG Group 2 n=20	FS PVF n=10	*CP PVF & REG n=13	
	Dry matter	Dry matter	Dry matter	Freeze dried	Freeze dried	Freeze dried	Freeze dried	Units
Calcium	1.12	0.65	0.9	1.09	0.64	0.72	1.36	g/100g
Magnesium	0.036	0.038	0.17	0.035	0.037	0.075	0.038	g/100g
Potassium	0.189	0.167	0.27	0.184	0.163	0.48	0.145	g/100g
Sodium	0.79	0.27	0.36	0.77	0.26	1.31	0.32	g/100g
Phosphorus	0.77	0.46	0.36	0.75	0.45	1.22	0.94	g/100g
Sulphur	0.81	0.55	0.42	0.79	0.54	0.71	0.49	g/100g
Iron	NA	NA	2.39	NA	NA	NA	NA	g/100g
Copper	16.9	16.4	8.9	16.4	16.1	3.6	4.8	mg/kg
Manganese	3.2	4.4	704	3.1	4.3	3.9	5.7	mg/kg
Zinc	27	17	78.1	26	16.2	46	35	mg/kg
Selenium	3.3	1.9	8.4	3.2	1.86	1.67	2.6	mg/kg
% DM or % FD	97.2	97.9		20.09	48.75			
Ca: Phos ratio	1.45	1.42	2.5	1.45	1.42	0.59	1.41	
Vitamin A	2865	2193	NA	2784	2146	3.13	2.48~	µg/100g
Vitamin E	38	42	NA	37	41	5.65	2.47~	mg/kg
Vitamin D3	≤ 0.05	≤ 0.05	NA	≤ 0.05	≤ 0.05	<0.03	≤ 0.05~	ug/g

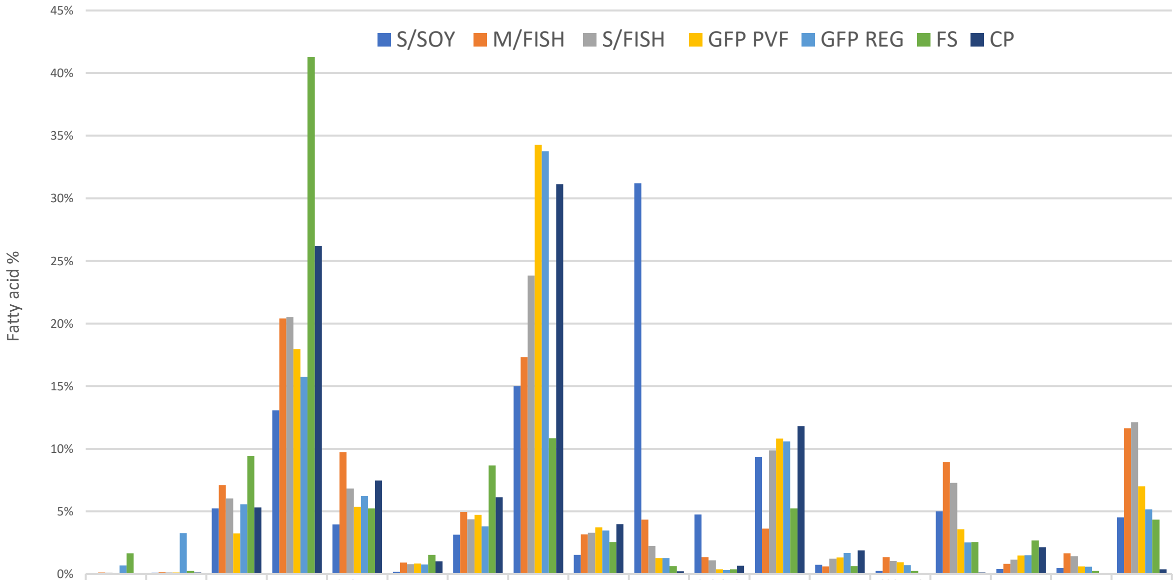
ND= Not detected, NA= not assessed, NR not reported.

*GFP Samples are reported on a freeze-dried and dry matter basis, so results would be directly comparable with Hendriks *et al* (2000) study and other species of petrel. Due to losses in freeze-drying, the CP samples will be underestimated, and converting to dry matter results was unreliable. The difference in freeze-dried and dry matter samples is unlikely to result in more than 5% variation between results (pers, comm Jackson, 2021).

~ Samples were reported as fresh samples rather than freeze-dried.

3.5.5 Comparing fatty acid composition of artificial translocation diets with the wild diet of three petrel species.

Many of the fatty acids were present only in tiny quantities in both wild and translocation dietary groups. In contrast, three main fatty acids (PAL, OLE, LIN) account for much of the variation in the dataset (Figure 41).



	C10:0 Capric	C12:0 Lauric	C14:0 Myristic	C16:0 Palmitic	C16:1n7 Palmitoleic	C17:0 Margaric	C18:0 Stearic	C18:1n9c Oleic	C18:1n7c Vaccenic	C18:2n6c Linoleic	C18:3n3 Alpha linolenic	C20:1n9 Eicosenoic	C22:1n9 Erucic	C20:4n6 Arachidonic	C20:5n3 Epa	C24:1n9 Nervonic	C22:5n3 DPA	C22:6n3 DHA
S/SOY	0.0%	0.1%	5.2%	13.1%	4.0%	0.2%	3.1%	15.0%	1.5%	31.2%	4.8%	9.4%	0.7%	0.2%	5.0%	0.4%	0.5%	4.5%
M/FISH	0.1%	0.2%	7.1%	20.4%	9.7%	0.9%	4.9%	17.3%	3.2%	4.3%	1.3%	3.6%	0.6%	1.3%	8.9%	0.8%	1.7%	11.6%
S/FISH	0.1%	0.1%	6.0%	20.5%	6.8%	0.8%	4.4%	23.8%	3.3%	2.2%	1.1%	9.9%	1.2%	1.0%	7.3%	1.1%	1.4%	12.1%
GFP PVF	0.0%	0.1%	3.2%	17.9%	5.4%	0.8%	4.7%	34.3%	3.7%	1.3%	0.4%	10.8%	1.3%	0.9%	3.6%	1.5%	0.6%	7.0%
GFP REG	0.7%	3.3%	5.6%	15.7%	6.2%	0.7%	3.8%	33.8%	3.5%	1.3%	0.3%	10.6%	1.7%	0.7%	2.5%	1.5%	0.6%	5.2%
FS	1.7%	0.3%	9.4%	41.3%	5.2%	1.5%	8.7%	10.8%	2.5%	0.6%	0.4%	5.2%	0.6%	0.3%	2.5%	2.7%	0.3%	4.3%
CP	0.0%	0.1%	5.3%	26.2%	7.5%	1.0%	6.1%	31.1%	4.0%	0.2%	0.6%	11.8%	1.9%	0.0%	0.1%	2.1%	0.0%	0.4%

Figure 41. Bar chart showing the percentage of fatty acids found in the three translocation diets (S/Soy, M/Fish, S/Fish), compared with the wild diet of Chatham Petrel (CP), fluttering shearwater (FS) and grey-faced petrel (GFP) collected by proventricular flushes (PVF) or regurgitations (REG) samples.

The table shows only fatty acids that were present in >1% of a diet. For the full dataset please see the supplementary material.

The three artificial diets consist of S/Soy=Brunswick tinned sardines in soya oil™, M/Fish=Mazuri fish analogue® in fish oil and S/Fish tinned sardines in spring water with fish oil added. The artificial diet and GFP samples were analysed from dry matter, while the FS and CP samples were from freeze-dried samples, and the results are expressed as a total % of fatty acids in the pooled sample. Pooled samples consist of two GFP diet samples group 1= 8 proventricular flushes (PVF) and group 2= 20 regurgitations (REG) samples. FS samples are from 10 pooled PVF. CP = 13 pooled samples (5 PVF and 8 REG).

The dominance of these three fatty acids relative to all 36 fatty acids is shown graphically in Figure 42, where the PCA graph shows euclidean distances distinctly separated by the prominence of PAL OLE and LIN. The S/Soy diet separates from all other wild and artificial diets due to markedly high LIN, ALIN and lower PAL and OLE. Conversely, all other diets had more elevated PAL and OLE with lower LIN and ALIN. This difference accounts for 39% of the variation seen in the data set (Figure 42).

Among the wild diet samples, the two GFP samples (PVF and REG) were the two most similar observations, while the FS were very distinct, suggesting that the sampling method (proventricular flush versus regurgitation) had less impact than the differences due to the different fatty acid composition of the species' diets (Figure 42, Figure 43, and Figure 44). FS PVF samples were much higher in PAL and lower in OLE than both the GFP samples, accounting for 27% of the variation in the data, while CP occupied middle ground with higher PAL like FS samples and OLE levels similar to GFP (Figure 42 and Figure 45).

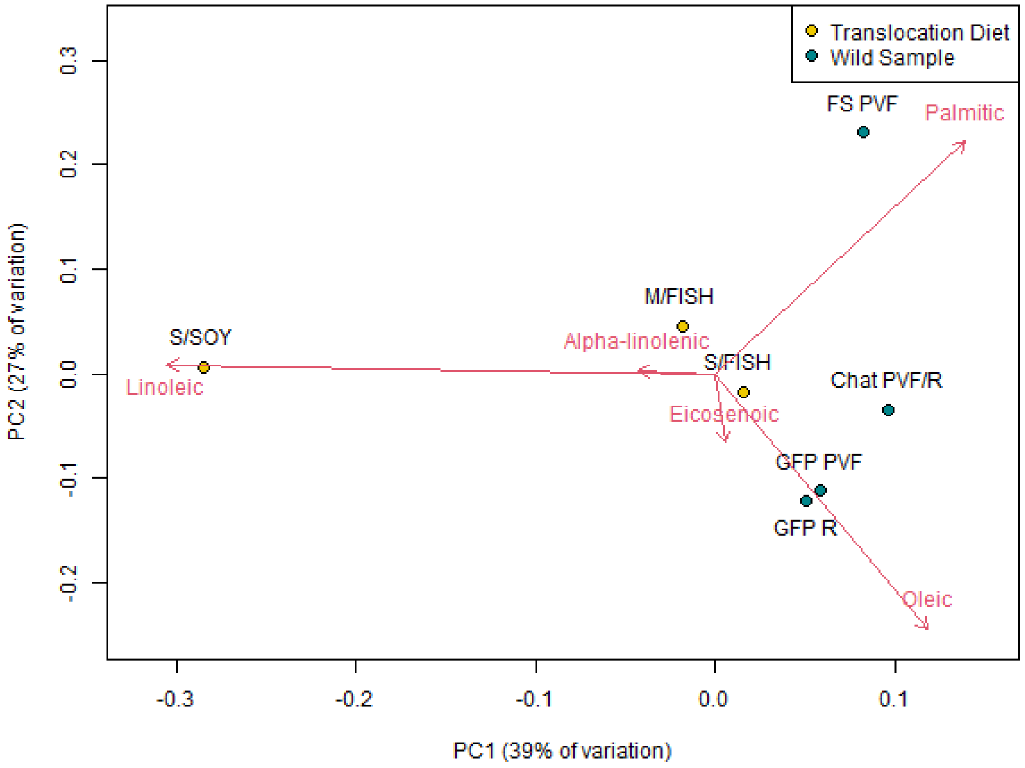


Figure 42. Principal component analysis graph showing the difference in fatty acid composition between three translocation diets (S/Soy, M/Fish, S/Fish) and three wild seabird dietary groups Chatham Petrel (CP), fluttering shearwater (FS) and grey-faced petrel (GFP) separated into groups based on sampling method, proventricular flushes (PVF) or regurgitations (R).

The fish oil supplemented diets (M/Fish and S/Fish) became more similar to each other when the very long-chain PUFA found in lower proportions in the diets were taken into consideration by transforming the data, which took the emphasis off the fatty acids which dominate the data set (LIN, PAL, OLE), (Figure 43). Suggesting that there are still subtle differences in the rare fatty acids between the wild samples and the artificial dietary groups, but the S/Soy dietary group is still clearly the most different from all other diets. DHA and EPA are found in the highest proportions in fish oil supplemented diets. Of the artificial dietary groups, the S/Fish diet is most similar to the GFP and CP wild diets (Figure 43).

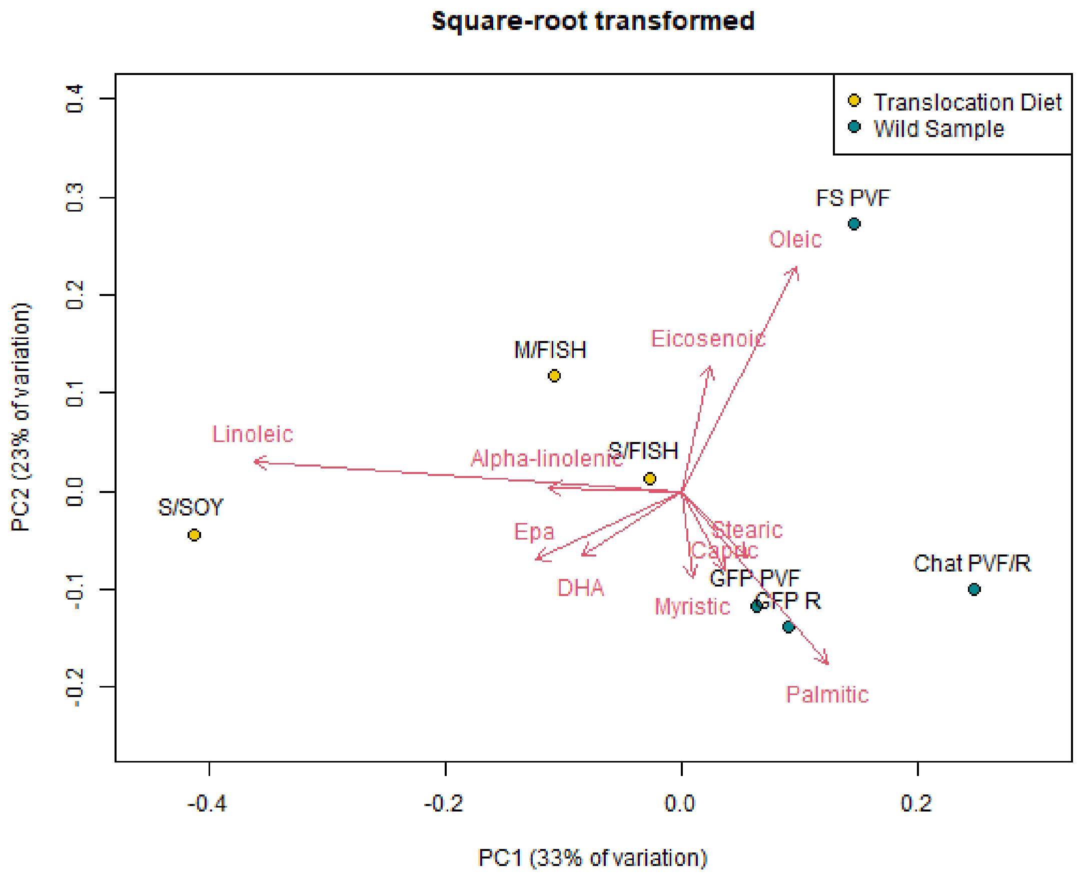


Figure 43. PCA of the fatty acid percentage in three translocation diets (*S/Soy, M/Fish, S/Fish*) and four groups of wild diet samples *(grey-faced petrel (GFP) proventricular flushes (PVF), GFP regurgitation (R), fluttering shearwater (FS) PVF and Chatham petrel (Chat) PVF/R)* compared using square root transformed data to highlight the influence of long-chain polyunsaturated fatty acids that are present in lower quantities.

Overall, the differences in sampling methods in the same species (GFP) produced very little difference in the relative proportions of fatty acid content compared to other species and artificial diets (Figure 41-45). Differences in fatty acid percentage between sampling methods averaged at 0%, with a maximum difference of 3.14% more lauric acid C12:0 in PVF samples and 2.12% more PAL in REG samples. The difference in sampling method may affect fatty acids present in smaller quantities more, as DHA and EPA were higher in PVF samples by 1.8% and 1.1%, respectively (Figure 44). However, these differences could simply be from individual differences in the groups of birds sampled.

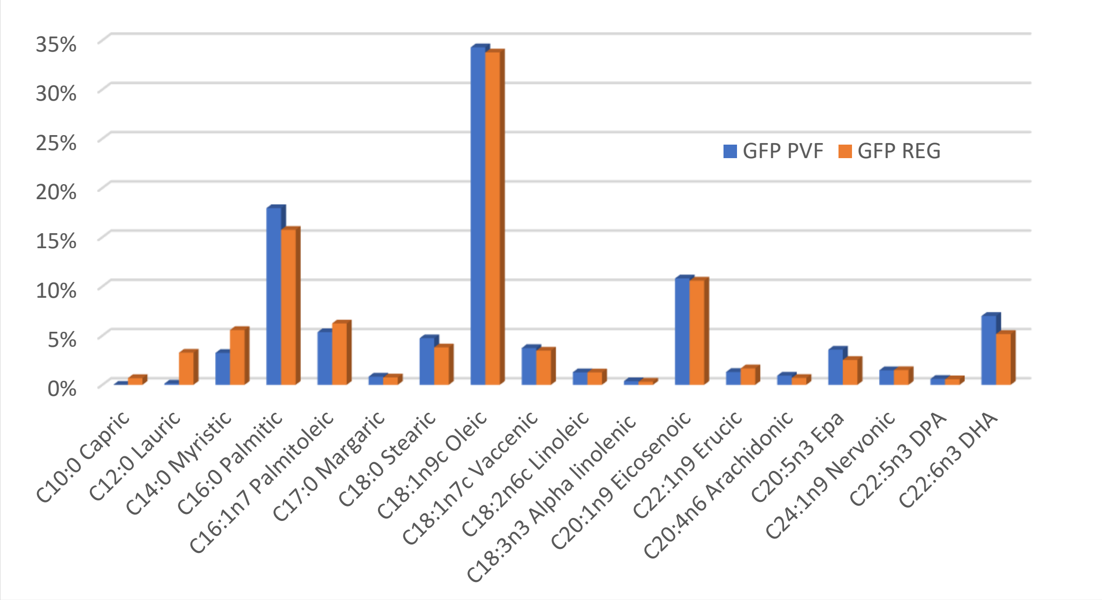
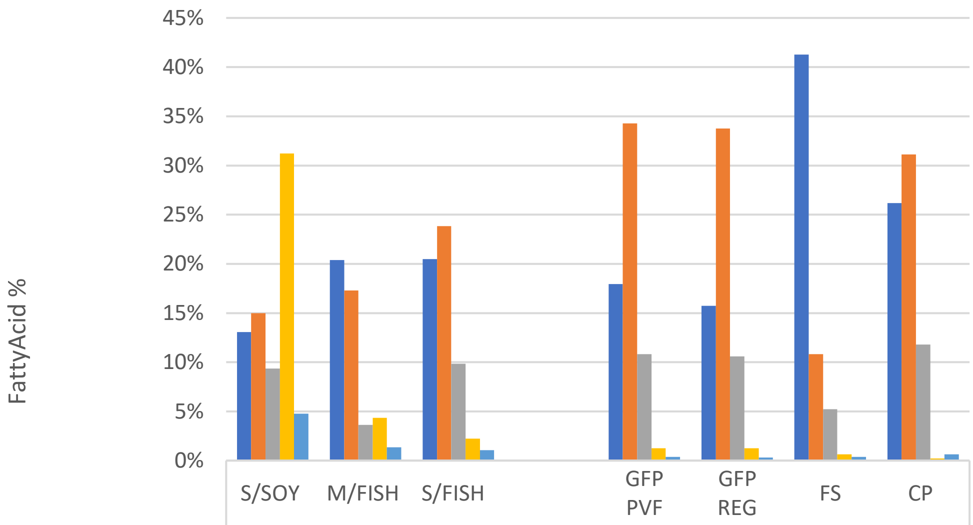


Figure 44. The difference in fatty acid percentages between grey-faced petrel (GFP) sampling methods proventricular flushing PVF and spontaneous regurgitation REG. Only fatty acids present at >1% are included in the graph.

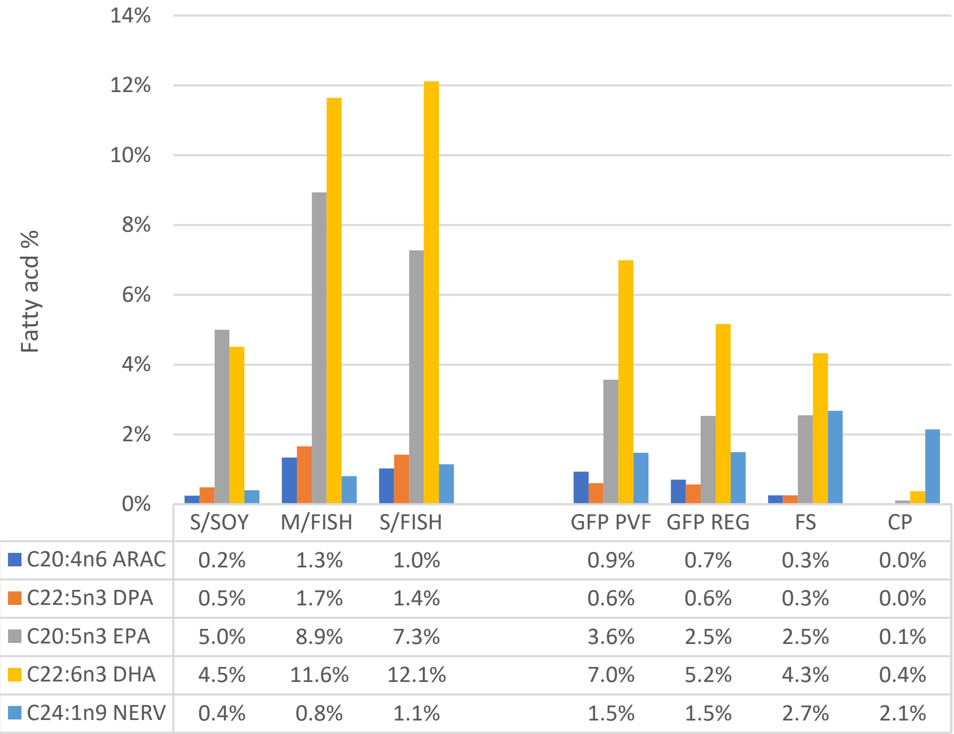
Individual fatty acid differences between diets

Individual fatty acids were varied and showed differences between wild and translocation dietary groups and between species. LIN was only prominent in the soya oil supplemented dietary group (S/Soy) (31%) and accounted for less than 1.3% of wild diets. The diet of the long strategy foragers (GFP and CP) featured OLE as the primary fatty acid (>30%) while short strategy foragers (FS) contained much less OLE (only 11% of the diet). FS groups were saturated in PAL, representing 41% of this species' fatty acids, double what was found in all other diets, except for CP, which had a slightly higher PAL of 26.2%. ALIN was minimally present in wild diets with <0.6% present in wild diets. These comparative differences of C16 and C18 fatty acids are outlined in Figure 45.



	S/SOY	M/FISH	S/FISH	GFP PVF	GFP REG	FS	CP
■ C16:0 Palmitic	13.1%	20.4%	20.5%	17.9%	15.7%	41.3%	26.2%
■ C18:1n9c Oleic	15.0%	17.3%	23.8%	34.3%	33.8%	10.8%	31.1%
■ C20:1n9 Eicosenoic	9.35%	3.63%	9.86%	10.81%	10.58%	5.22%	11.80%
■ C18:2n6c Linoleic	31.2%	4.3%	2.2%	1.3%	1.3%	0.6%	0.2%
■ C18:3n3 Alpha linolenic	4.8%	1.3%	1.1%	0.4%	0.3%	0.4%	0.6%

Figure 45. Differences in predominant fatty acid percentages between three translocation diets and pooled proventricular samples from three species of seabird (GFP, FS and CP). GFP were sampled by proventricular flushing (PVF) or regurgitation (REG).

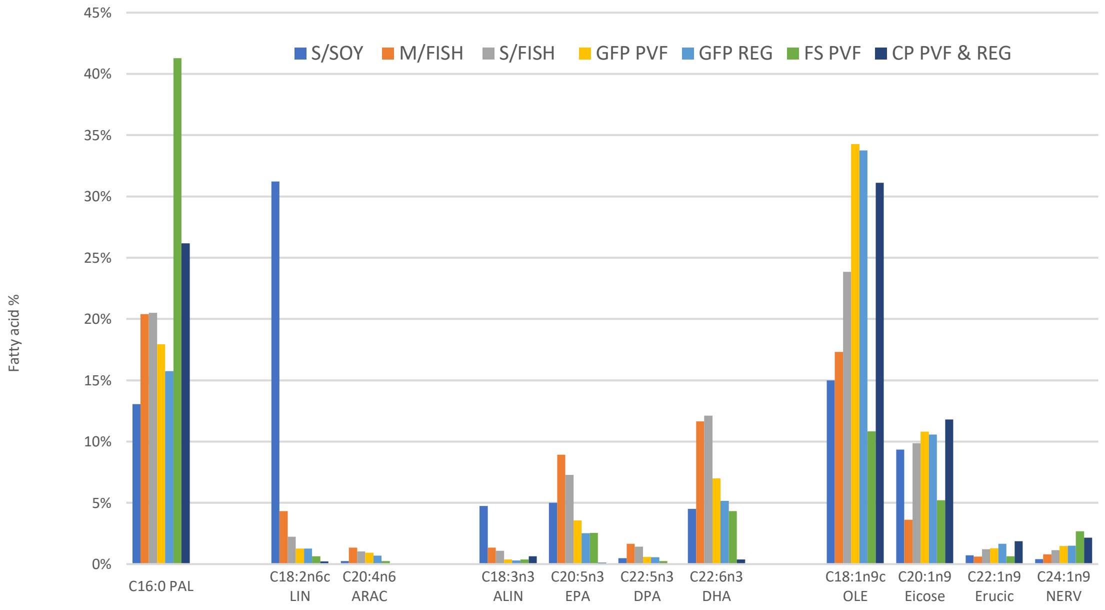


	S/SOY	M/FISH	S/FISH	GFP PVF	GFP REG	FS	CP
■ C20:4n6 ARAC	0.2%	1.3%	1.0%	0.9%	0.7%	0.3%	0.0%
■ C22:5n3 DPA	0.5%	1.7%	1.4%	0.6%	0.6%	0.3%	0.0%
■ C20:5n3 EPA	5.0%	8.9%	7.3%	3.6%	2.5%	2.5%	0.1%
■ C22:6n3 DHA	4.5%	11.6%	12.1%	7.0%	5.2%	4.3%	0.4%
■ C24:1n9 NERV	0.4%	0.8%	1.1%	1.5%	1.5%	2.7%	2.1%

Figure 46. The very long-chain polyunsaturated fatty acid percentages of three different translocation dietary groups and pooled wild diet samples from three species of petrels (grey-faced petrel, fluttering shearwaters and Chatham petrels). Grey-faced petrels were sampled by proventricular flushing (PVF) or regurgitation (REG).

CP fatty acid profiles were uniquely different to the other seabirds and the artificial diets. In CP samples, DHA was noticeable low compared to other dietary groups, 0.4%, even though DHA was the second-highest very long-chain PUFA present in CP samples. DPA and ARAC were not detected in CP samples. NERV was the only very long-chain PUFA found in high proportions, and overall the CP profile showed a predominance of omega n-9 precursors for NERV (OLE, eicosenoic acid C20:1n9 and erucic acid C22:1n9) (Figure 45, Figure 46, Figure 47). The omega n-9 precursors were found in similar proportions in GFP diets. NERV was found in the highest amount in FS diets which also had the lowest amount of n-9 precursors. No translocation diet contained similarly high levels of nervonic acid as the wild diets, nor its n-9 precursors (OLE, eicosenoic acid C20:1n9 and erucic acid C22:1n9).

Fish oil supplemented translocation diets contained enough ARAC, DPA, EPA and DHA, to meet and exceed the same dietary percentages seen in the wild diets of all three species. The S/Soy diet was lower in ARAC (0.5-0.7%) and DHA (0.7-2.5%) than what was consumed by GFP in wild dietary samples. The S/Soy diet had similar proportions of EPA, DPA and ARAC to match consumption in all other species. All translocation diets contained more LIN and ALIN than wild diets, but only the S/Soy diet had abundant amounts present.



	C16:0 PAL	C18:2n6c LIN	C20:4n6 ARAC	C18:3n3 ALIN	C20:5n3 EPA	C22:5n3 DPA	C22:6n3 DHA	C18:1n9c OLE	C20:1n9 Eicose	C22:1n9 Erucic	C24:1n9 NERV
S/SOY	13.1%	31.2%	0.2%	4.8%	5.0%	0.5%	4.5%	15.0%	9.4%	0.7%	0.4%
M/FISH	20.4%	4.3%	1.3%	1.3%	8.9%	1.7%	11.6%	17.3%	3.6%	0.6%	0.8%
S/FISH	20.5%	2.2%	1.0%	1.1%	7.3%	1.4%	12.1%	23.8%	9.9%	1.2%	1.1%
GFP PVF	17.9%	1.3%	0.9%	0.4%	3.6%	0.6%	7.0%	34.3%	10.8%	1.3%	1.5%
GFP REG	15.7%	1.3%	0.7%	0.3%	2.5%	0.6%	5.2%	33.8%	10.6%	1.7%	1.5%
FS PVF	41.3%	0.6%	0.3%	0.4%	2.5%	0.3%	4.3%	10.8%	5.2%	0.6%	2.7%
CP PVF & REG	26.2%	0.2%	0.0%	0.6%	0.1%	0.0%	0.4%	31.1%	11.8%	1.9%	2.1%

Figure 47. Percentages of fatty acids in three translocation diets (S/Soy, M/Fish and S/Fish) and the proventricular contents of three petrel species comparing palmitic acid with the highest percentages of omega n-6, n-3, and n-9 fatty acids. The species sampled were grey-faced petrels (GFP), fluttering shearwaters (FS) and Chatham petrels (CP). Samples were collected by proventricular flushing (PVF) and or spontaneous regurgitation (REG).

3.6 Discussion

This study aimed to compare the wild diet of three New Zealand burrow-nesting seabirds with the S/Soy translocation diet and two alternative diets that used fish oil supplements. The results indicate that the wild seabird diets vary significantly from translocation diets based on soya oil, particularly concerning the composition of the fatty acid profiles. The method of sample collection can significantly affect results, especially regarding ash and fat content, and future studies need to consider the timing of sampling as this is likely to be a source of variation in results. Differences between species and foraging strategies were still apparent irrespective of sampling methods.

3.6.1 Replicating wild diets: the effect of sampling methods proximate analysis.

To formulate an artificial version of a wild diet for managing wildlife species, the basic analysis of nutrient components must be accurate. This study showed that the method of collecting proventricular content had a significant effect on the proximate analysis results in GFP. Studies investigating wild diets need to consider the sampling method, specifically that proventricular flushing can produce higher ash, protein, and moisture percentages. Collecting samples by spontaneous regurgitation reported significantly higher fat and dry matter percentages in both total and residual amounts. Sodium was higher in PVF, which might be explained by the composition of the flushing solution.

Several of the sample differences are expected based on the sampling method itself. The changes in moisture content are not unusual since the PVF sampling method adds fluid to the proventriculus for flushing, reducing the total dry matter of the sample. This finding was repeated within the same chicks, at the same colony and between geographical locations. It is possible the sodium content was artificially elevated by using isotonic saline (0.9% sodium chloride) to flush the proventriculus, or that the bile salt, which is naturally high in seabirds (Place, 1992), may be flushed out of the gizzard. Ash was highly variable and increased in PVF samples, possibly from flushing non-digestible items out of the gizzard. In seabirds that spontaneously regurgitate for defence (Foster et al., 2010), the proportions of ingesta regurgitated may vary. REG samples may retain the mineral content located deeper in the gizzard, and eject the more superficial layers of oil in the proventriculus as an anti-predator defence which will be discussed in detail in the section below.

To identify causes of variation, it helps to consider sources of similarity. Different sampling methods within the same chick did not affect the proportion of fat, protein, and ash. However, this finding needs more testing to be reliable, as the sample size of n=3 will not be powerful enough to determine a difference. Chick selection in these three chicks was biased, as all appeared to be recently fed, based on their weight and palpably full proventriculus. If accurate, this finding indicates that the sampling method may be less important in recently fed chicks.

The geographical location of sampling did not affect the composition of fatty acids samples within or between distant colonies of GFP. In contrast, the comparison of sampling methods within the same colony demonstrated differences attributed to the aforementioned predictable changes from the sampling method itself, (ie PVF higher moisture content, lower total dry matter, and higher ash).

The similarity in diets within and between distant colony sites indicates that GFP foraging is relatively constant over the ‘finishing’ stage of the diet, consistent with previous findings in this species (Hendriks et al., 2000). Diet content may vary greatly, and each feed represents only a brief picture in time over what is likely to be a highly variable diet based on parental foraging success. The wide range of proximate analysis results may also reflect limitations in the sampling method, potentially from samples being collected at different stages of digestion, unequal volumes of samples being pooled, and small sample volumes being highly variable and therefore may not be representative of larger sample volumes.

Compared to other species, the fat content of samples from daily foraging species (FS) was lower in fat and higher in protein content than long strategy foragers (CP and GFP). The GFP diet has previously been studied as a model for the rarer seabirds, including the Chatham island petrel (*Pterodroma magentae*; tāiko) and CP. In the present study, GFP and CP diets were only similar to PVF samples, not REG samples in which the fat proportions were high.

Digestion and fat variation from sampling method

This study found that fat levels are likely to be overestimated in regurgitation samples compared to proventricular flushing. The sampling method produced significantly different proportions of oil; however, the fatty acid proportions in the oil were only marginally affected. If the aim is to compare the relative proportions of fatty acids present in the oil between species and translocation dietary groups, then either PVF or REG could be used. However, the absolute volume of fat that needs to be present in a translocation diet needs further refinement.

Improved sampling timing is recommended in future studies to avoid differences in fat volumes due to digestion times. With the exception of diving petrels, the oils fed to

Procellariiformes chicks are not secretory products but rather concentrated dietary lipids regulated by the amount of lipid emptying from the pylorus. The anatomy and location of the pylorus are adapted to retain lipids while allowing water-soluble ingesta to pass through preferentially. In addition, lipid emulsifiers from the intestine are refluxed retrograde into the pylorus and are believed to assist in gradual lipid metabolism before it reaches the intestine (Padilla, 2015). REG samples from chicks take advantage of the regurgitation that takes place in most Procellariiformes upon handling, with some species able to forcefully expel gastric oil as a defence mechanism (Padilla, 2015). The higher fat content in regurgitation samples from GFP is likely a reflection of fractioned lipid digestion and preferential expelling of lipid content, and results of the dietary analysis could be improved by sampling the chicks as close to being fed by the parent as possible. The fat content did not differ in CP diets in this study; however, the loss of oil during the laboratory analysis and the small sample size makes this finding unreliable.

Some variation in oil content may be related to different age ranges, as generally, the amount of stomach oil in most species of petrel chicks declines as they near fledging (Warham, 1996). However, this may not be consistent between species. For example, in a previous study on Leach's storm petrel, the amount of proventricular oil was highly variable between all chick age groups and, in contrast, increased during the period of pre-fledging weight loss (Place et al., 1991). The volume of oil in older Leach's storm petrel chicks was dynamic, changing in response to dietary intake and the accumulation of oil was due to slower gastric emptying (Place *et al*., 1989 as cited by Place *et al., 1991)*.

Ash analysis

The ash content in diet samples is an important consideration as a measure of vitamins and minerals (Sinclair et al., 2015). Ash provides an approximate estimate of total inorganic matter, and it is needed to calculate carbohydrates by difference. The proportion of ash was relatively high in all proventricular samples. It is likely that proventricular flushing is more effectively dislodging indigestible fragments trapped in the gizzard, such as bone, cartilage, chitin (hard exoskeletons) and squid beaks that are sporadically egested by seabirds.

A potential source of high ash in Hendricks *et al.* (2000) study was from scooping soil into the samples, particularly elevating the iron levels via the inclusion of titanomagnetite sand prevalent in the area. Therefore, proportions of manganese, zinc and selenium in Hendricks et al. (2000) may also be higher due to soil contamination. Care was taken in the present study to avoid any substrate contamination, and the ash content was still elevated in multiple chicks.

Cephalopods can also be a rich source of essential elements such as phosphorus, manganese, zinc and copper, with sulphur present in lower concentrations. (Lourenço *et al*., 2009). However, the ingestion of soil and feathers by chicks in the burrow cannot be ruled out. Given the steps taken to reduce soil contamination, the mineral analysis from the present study should be considered a more reliable reflection of wild dietary intake than the previous study of Hendricks (*et al*. 2000). A potential consideration is the refluxing of squid beaks; however, some authors suggest that squid beaks may not contribute significantly to mineral content. This is based on studies in Humboldt squid (*Dosidicus gigas*) which show that squid beaks are a biocomposite made up solely of organic compounds, chitin and proteins and do not contain any hard minerals such as calcium phosphate or carbonate. (Cai *et al*., 2017). Although there are minerals in chitin, it is uncertain how digestible these are.

Due to the heterogeneity of the composition of diet samples in this study, future nutritional studies should consider separating the indigestible hard fragments for separate proximate analysis to remove the effect of the significant variation ash can produce using these sampling methods. Stomach flushing methods that remove grit have been used successfully in passerines, but the need for a general anaesthetic to collect samples makes this method more invasive and impractical (Gionfriddo et al., 1995).

GFP diet comparison to previous studies.

Hendriks *et al*. (2000) analysed the nutrient composition of 43 regurgitation samples from similar aged GFP chicks at the same Te Henga colony in 1997 (Hendriks *et al*., 2000). When fat and protein results of previous REG samples were compared to PVF samples in this study, the results were similar. However, the REG samples in the present study showed significantly higher fat and lower protein. The difference in part may be explained by the sampling method. A key difference in the previous study was that if the beginning of regurgitation was missed, it was scooped off the ground, so regurgitation samples are likely to have included the complete voided sample. However, this method led to soil contamination, which also may have artificially elevated the ash component of the analysis and thereby decreased other fractions of the analysis.

Any direct comparisons with Hendricks *et al*. (2000) study needs to consider the difference in pooling method, chick age and digestion time. In the previous study, only one proximate analysis was run on the 43 samples pooled together, so the variation inherent in the sampling method and range in the diet would be hard to capture by the mean alone. Since the proximate analysis is used to ascertain the recommended level of protein and oil in an artificial diet, the mean value may easily result in an under or overestimation of the actual oil requirement. The age of the chicks sampled in Hendricks *et al* (2000) study (60-90 days) was younger than the groups sampled in this study; depending on which average fledging age you consider, weather is it the 118 days reported by Imber (1976) or 140 days by Dunn (2012). However, this pre-fledging age range is less likely to cause a difference, as Hendricks *et al*. (2000) also reported no significant difference in diet composition between chicks aged greater than 10 days old.

3.6.2 Differences in fatty acids between wild and artificial translocation diets

Overall, no current translocation diet for burrow-nesting petrels in New Zealand that has been studied adequately replicates the PAL and OLE proportions present in the wild diets of GFP, CP and FS. Diets supplemented with fish oil, particularly the S/Fish diet, reach a closer approximation of the wild seabird diets in the present study. The soya oil portion in the S/Soy diet dilutes proportions of all other fatty acids with LIN and ALIN, to volumes lower than are found in the S/Fish diet. The significance of this dilution effect is that longer chain PUFA ARAC and DHA are fed at lower proportions than found in the wild diet of GFP. Diet samples from wild FS chicks had similar proportions of DHA, DPA and ARAC to the S/Soy diet. As it is unknown if seabirds can efficiently transform ALIN and LIN into very long-chain PUFA (ARAC, EPA, DPH and DHA), the value of LIN being present in such high volumes in the S/Soy diet is unknown. However, studies in humans and chickens (*Gallus gallus*) have shown that even in a species that can desaturate and elongate LIN and ALIN into long-chain PUFAs, it is more beneficial and energy-efficient to feed very long-chain PUFA (ARAC, EPA, DHA) in the diet than LIN (Burdge & Calder, 2005; Gerster, 1998; Koppenol et al., 2014). Due to the high availability of ARAC in their natural diet, many carnivorous mammals and fish have lost the capacity to synthesise it, so it is an essential dietary requirement (Klasing, 1998). Therefore it should not be assumed that all avian species are able to synthesise all of the PUFA that they metabolically require, and a prudent translocation diet would supplement ARAC and DHA (Klasing, 1998). This study shows that in GFP, birds fed artificial diets with soy oil do not have similar proportions of ARAC when compared to wild samples, suggesting that ARAC may need to be supplemented as we do not know if they have the capacity to synthesise it.

The benefit of feeding OLE and PAL is that it is well established as the predominant part of wild seabird diets. In birds, both OLE and palmitoleic C16:1n7 cis-9 are common due to the large amount of monosaturated fatty acids in the diet and the ability to actively synthesize these fatty acids within the bird. (Klasing, 1998). The predominance of OLE, PAL and Palmitoleic acid in proventricular oil is also a common finding for seabirds; in a study of 5 different species of

Procellariiformes, these three fatty acids were the most predominant saturated fatty acid found in proventricular samples[5] (Connan et al., 2005).

It is unlikely that seabird fatty acid synthesis is as efficient as domestic species since their wild diet is rich in very long-chain PUFA. The ability of birds to synthesize fatty acids can be relative to the level of dietary fat; for example, some species of faunivores (such as common murres) have a relatively low capacity for fatty acid synthesis by the liver compared to granivores since a high rate of de novo synthesis of fatty acids in unnecessary given their OLE and palmitoleic rich diet (Herzberg & Rogerson, 1990; Klasing, 1998). In avian marine carnivores, PAL and OLE are the predominant fatty acid, with LIN present only in minimal levels compared to avian herbivores, insectivores and omnivores (Potter et al., 2013). Demonstrating how well seabirds are adapted to surviving with low levels of LIN, observed in all the wild diets.

LIN and ALIN can only be synthesized by plants and can only be sourced from the diet; they are therefore considered essential dietary fatty acids in many species of birds. In domestic species, supplementation of LIN and ALIN has proven benefits as a precursor to synthesizing very long-chain PUFAs. The effectiveness of supplementing LIN and ALIN in a diet is questionable when the option of providing a fish oil-rich diet with already synthesized very long-chain PUFA is a feasible alternative.

Very long-chain PUFA appear to be unique to each species; therefore, a fish oil supplement high in DHA and ARAC and a NERV supplemented diet is likely to be the safest approach to a translocation diet that encompasses unknown nutritional requirements. Preloading seabird chicks with drastically different fatty acids from their wild diet may negatively affect brain development. NERV is an exceptionally long PUFA that plays a vital role in developing and maintaining the brain and the biosynthesis and improvement of nerve cells in mammals (Li et al., 2019). It is an important functional component of the sphingolipids of white matter and myelinated nerve fibres (Li *et al*., 2019). No artificial translocation diet replicated the higher proportions of NERV found in all the wild seabird diets studied, with S/Soy showing only minimal amounts (0.4%) and S/Fish being the closest approximation (1.1%). Samples analysed from FS and CP showed higher amounts of NERV (2.7% and 2.1%) than GFP (1.5%), which may represent a specific foraging item in the diet. In the case of the CP, the proportion of NERV may be the end product of more n9 precursors in the diet (OLE 31.1%, eicosenoic acid C20:1n9

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[5] The 5 speicies included short-tailed shearwaters *Puffinus tenuirostris*, white-chinned petrels *Procellaria aequinoctialis*, blue petrels *Halobaena caerulea*, thin-billed prions *Pachyptila belcheri*, and Antarctic prions *Pachyptila desolata*

11.8% and erucic acid C22:1n9 1.9%) with minimal amounts of n3 and n6 precursors compared to the other species sampled. Domestic species of birds, such as poultry, can synthesize the n-9 family of fatty acids (de novo), so these fatty acids are not required in the diet (Klasing, 1998). However, the efficiency of fatty acid synthesis in seabirds is still unknown, and given that domestic species still need to synthesis n-9 fatty acids, the availability of precursor fatty acids is likely to be necessary. One of the only published feeding trials of the Mazuri fish analogue diet® (the M/Fish diet without fish oil supplementation) was in Beluga whales, where a reduction in plasma levels of NERV was found after three weeks compared to a wild piscivorous diets (Mazzaro *et al*., 2011). Based on this finding, the authors recommended that NERV acid was also supplemented into the captive diet of Beluga whales (Mazzaro *et al*., 2011).

3.6.3 Future studies

Future assessments of translocation diets in these species would benefit from comparison with the vitamin and amino acid analysis that has been included in the supplementary material of this chapter. Vitamin D3 was not detected in high levels in any wild seabird diet, however, assessment of vitamin D2 would be worth investigating further.

3.6.4 Summary

This study aimed to compare the wild diet of three New Zealand species of burrow nesting seabirds with the translocation diet based on sardines in soya oil and two alternatives that used fish oil supplements. Overall, the results indicate that the wild diets vary significantly from the translocation diets based on sardines in soya oil, particularly the composition of fatty acid profiles. Sampling methods are very important to determine accurate proportions of nutrients that should be fed in a wild diet, and this study outlines many of the limitations faced in determining normal dietary reference ranges in wild species. The variety in proximate analysis results outlines the importance of using stringent sampling protocols that consider digestion times, variable ash content and avoiding sample contamination. Establishing reliable wild diet parameters appropriate to the seabird species being translocated is unlikely to be achieved soon, given the cost, sample sizes and practical limitations of such studies. Therefore, a prudent approach would be to consider varying the levels of fat content in the artificial diet using oils fortified with ARAC, DHA and NERV, to reflect better those found in the wild diets of the respective species. Substituting soya oil with fish oil will provide a diet closer to the composition of the wild diet in the three species studied, and the fish oil may benefit from further NERV supplementation. It is important to note that the aim is to feed the artificial translocation diets only as a ‘finishing diet’, and my results support transitioning fledging chicks onto a wild diet as soon as possible, as this will also help to increase variety in the diet fed and buffer any nutritional limitations that may be undetected at fledging.

Chapter Four

General Discussion

4

4.1 Summary of Findings

Proventricular oil is a vital part of Procellariiformes diets[6]. This study investigates the effect feeding fish or soya oil has on translocated seabird chicks. As the nutritional requirements of seabirds are still unknown (Speer, 2015), feeding a diet similar to wild diets is the safest option until more is known about the absolute nutritional requirements of Procellariiformes. This study found that the S/Soy translocation diet contains fatty acid ratios that are substantially different to wild-fed chick proventricular oil samples in all three species studied (CP, FS and GFP). When chicks were fed the S/Soy diet for 2-3 weeks, RBC membrane fatty acid in both GFP and FS built up higher proportions of LIN, a plant-based C-18 fatty acid; while at the same time, reducing important very long-chain PUFAs, (DHA, DPA and ARAC), compared to fish oil supplemented and wild dietary groups. Fish-oil supplemented diets had the reverse effect and were more like wild-type diets, producing RBC fatty acid ratios that resembled recently-transferred FS and wild GFP colony samples more closely. DHA and ARAC maintained higher levels in fish oil supplemented feeding trials, while EPA was minimally affected. No diet supplemented enough NERV to match the levels found in the proventricular samples of wild seabirds. NERV and two other omega n-9 fatty acids were the most predominant very long-chain PUFA in the critically endangered CP chick diet samples.

Dietary trials showed that despite soya oil dramatically changing the RBC membrane PUFA proportions, there was no major detrimental effect on the success of the FS chicks returning to the colony compared to fish oil, fed chicks. This limited impact may be due to the hangover-effect of parental feeding and the likelihood that birds quickly re-establish PUFA proportions once they begin foraging for themselves. Chicks that were fed large meals by parent-birds before the transfer had fatty acid proportions similar to wild fed and fish oil dietary groups, even if they were fed the S/Soy diet. Fledging parameters were similar between the dietary groups, except for in the S/Soy fed GFP chicks, which gained significantly higher weights.

Deaths due to visceral gout occurred in GFP chicks irrespective of the diet fed. Supplementing translocation diets with fish oil improved fledging RBC fatty acid ratios compared to soya-oil fed chicks, reaching levels that resemble the fatty acid ratios of wild fed or recently transferred seabirds.

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[6] With the exception of diving petrels.

Given the gap in knowledge of nutrient requirements in seabirds, studies of wild diets that measure 'as fed' proportions of nutrients are important to approximate the likely nutritional requirements of seabirds. This study assessed the effect that different methods of collecting proventricular samples had by analysing proximate analysis results of PVF and REG samples in three species of seabirds (CP, GFP and FS). A limitation of the sampling methods used was that REG samples reported higher fat and lower ash content than PVF samples, which should be taken into consideration when interpreting results.

Irrespective of the sampling method, short foraging strategy species (FS) showed higher protein and lower fat content when compared with long foraging strategy species (CP and GFP). Comparing proventricular oil fatty acid ratios from wild chicks to the fatty acid composition of the S/Soy diet showed similar findings to the RBC fatty acid comparisons, with significantly higher LIN and ALIN proportions only present in the S/Soy diet. This build-up of LIN and ALIN distinctly sets the S/Soy diet apart from all other wild proventricular oil samples and the fish oil supplemented dietary groups. DHA and ARAC were lower in the S/Soy diet than in fish oil supplemented diets and in the GFP and FS wild samples. OLE and PAL were the predominant fatty acids in wild diets and showed species-specific differences. No artificial diet provided sufficient NERV to reach levels in wild diets, with sardines in fish oil being the closest alternative.

Wild FS and CP proventricular oil samples had relatively elevated NERV and omega n-9 precursors (OLE, C20:1n9 eicosenoic acid and C22:1n9 erucic acid). CP had markedly low DHA, DPA, EPA and ARAC levels in comparison to other species wild diets and the artificial translocation diets. Supporting the hypothesis that species-specific dietary requirements are likely to exist and that the S/Soy diet will need refinement to cover all species translocated. In all species studied, adding fish oil to the artificial diet will increase the similarity of the fatty acid proportion of the diet to the wild diet.

Given the importance of very long-chain PUFA in diet and the know benefits for health and immunity and chick development (Korver & Klasing, 1997; Pike, 1999) a prudent diet would include supplementation with DHA, ARAC and NERV fortified oils, as the efficiency of synthesising them from LIN and ALIN is unknown. The volume and proportion of fat-fed in the diet need further investigation, carefully considering the sampling methods used to determine normal fat levels within the diet.

4.2 The Importance of fatty acid incorporation into the diets

The health benefits of long-chain PUFA in diets are widely known. (Klasing, 1998; Pike, 1999). There are two primary sources of DHA in avian diets, either by consuming ALIN from plant or seed-based diets and using it as a precursor for DHA synthesis or by consuming DHA directly from marine algae or in seafood with highly concentrated DHA (Cherian, 2015). The key benefit of providing LIN and ALIN in the diet is that these fatty acids are the precursors for forming DHA, ARAC and EPA in the diet. However, in seabirds that are obligate piscivores, these plant-based fatty acids may not be metabolizable to create DHA, EPA and ARAC, since PUFA are naturally found in high proportions in their wild diet (Klasing, 1998). Feeding DHA and EPA directly in food has proven to be more effective than providing ALIN and LIN directly in avian species that can metabolise them, as the conversion rate is can be low due to the competitive enzymes involved (Cherian, 2015; Koppenol et al., 2014; Pike, 1999). EPA an omega n-3 fatty acid was not affected by diets fed in this study, while ARAC and DHA were significantly reduced on the S/Soy diet. Omega n-3 and omega n-6 PUFA such as DHA and ARAC are abundant in the avian central nervous system and makes up 10-15% of the total lipids in the central nervous system of newly hatched chickens (Cherian, 2015). Deficiencies in DHA and ARAC can detrimentally affect immunity, inflammatory responses of eicosanoids, tissue repair and organ development (Speake & Deans, 2004) and aerobic potential of muscles during flight (Hulbert & Abbott, 2012). DHA is vital for the development of brain, retina and cardiac tissue. In many species, DHA is crucial in early development, as deficiencies during growth have been shown to have detrimental effects on brain, cardiac and retinal development, decreasing visual acuity, cognitive development and resulting in a higher incidence of coronary heart disease and inflammatory autoimmune disorders (Pike, 1999).

4.3 Carry-over effect of the wild diet

Translocating chicks that have recently been parent-fed is likely to have a carry-over effect on their RBC membrane fatty acid proportions. The heaviest GFP chick in the S/Soy diet group had recently been fed by parents before transfer and showed RBC’s fatty acid ratios that were far more similar to the wild diets than all the other S/Soy fed chicks near fledging. Translocation protocols recommend assessing if chicks have recently had a parental meal before transfer and the feeding frequency be adjusted to account for their most recent meal (Gummer, Taylor, et al., 2014a). Selecting chicks at transfer that have recently been fed are likely to have the benefit of parent foraged proventricular oils for longer than less recently fed chicks and this has the potential to buffer the effects of feeding soya oil, preventing a build-up of LIN and ALIN at the expense of DHA, ARAC and OLE. Further assessment of RBC fatty acid changes and return rates would be beneficial to confirm this finding, as the sample sizes are small. Transporting chicks with a full proventriculus will have a higher risk of regurgitation; however, FS chicks in this transfer have returned as adults despite regurgitating in the transport box as chicks.

4.4 Translocation diet review

Feeding fish oil supplemented diets produces RBC membrane fatty acid proportions that are more similar to wild diets irrespective of whether the base of the diet is tinned sardines in water or Mazuri fish analogue®. GFP chicks fledged at weights closer to preferred weights on M/Fish diet, however, it is unclear if this is an effect of the fish oil or the Mazuri fish analogue® diet in GFP. Feeding the Mazuri fish analogue® diet was practical on-site as it did not require an electric blender and electricity to prepare. However, the cost, limited availability in New Zealand and short expiry date made it an impractical alternative to the S/Soy diet.

The addition of fish oil to diets increases expense, has a shorter expiry date than cheaper oils and needs to be stored appropriately to reduce the risk of rancidity. This study shows that feeding fish oil will result in fatty acid incorporation into tissues that are closer to the natural diet, with higher levels of very-long-chain PUFA (greater than C20 carbon), which has proven health benefits in other species (Korver & Klasing, 1997; Pike, 1999). However, the benefit of RBC's membranes being fortified with long-chain PUFA (DHA, ARAC and DPA) did not translate into significantly improved return rates for FS to the colony. Daily foraging in the wild is likely to rapidly transition FS back onto the wild diet after fledging. However, the effects of supplementing fish oil to long foraging species GFP and CP, are yet to be established with return rates.

4.5 Fat proportions in the diet.

This study has identified the importance of the type of oils used in the diet by directly measuring the effect on the composition of RBC phospholipid membrane fatty acids. The membrane phospholipid composition directly impacts the inflammatory responses, immunity, and health of cardiac, retina and neuronal tissue (Pike 1999). The kind of fatty acids that mimic the wild diet have been identified, however, the exact volume of fat required to be fed in the diet is still unknown. Based on proximate analysis results, there is a wide variety of possibilities from 20 to 60% in GFP. However, several limitations in the collection of the proximate analysis samples make it difficult to establish whether these findings are accurate. In particular: the variability in ash content, digestion times, pooling unequal volumes of samples, and in CP the small sample sizes with losses during the freeze-drying process at the lab. The same ratio of oil to tinned sardines or Mazuri fish analogue® was fed to all species, although it is clear that FS samples, the only species daily feeding species, produced less oil than CP or GFP. Some species like diving petrels are unlikely to need similar levels of proventricular oil as it is not in their wild diet. Given that long foraging species of Procellariiformes are adapted to variable provisioning of food (Dunn 2010), it may be that this species is more resilient to varying fat volumes in artificial diets. As proventricular oil is a source of hydration, lack of oil may predispose birds to conditions such as gout, and therefore establishing the optimal volumes of oil to feed would be beneficial. However, volumes of oil required may vary at different life stages, and in rehabilitation settings, the volume of oil is reduced intentionally in seabirds near release as the faecal consistency of birds becomes too oily and is at risk of ruining the waterproofing of the mature plumage.

4.6 Nutritional theory of Procellaiiforme diets.

The purpose of exceptionally high-fat stores accumulated by petrel chicks has been widely questioned. It may be so that seabirds can exploit highly variable and unpredictable food resources, unevenly distributed at sea, providing insurance against poor feeding conditions that may lead to periods of fasting or delivery of food being stopped altogether (Gebczynski & Jadwiszczak, 2000). Other possibilities include that the high-fat deposition is a by-product of a fat-rich and nutrient-poor diet (Ricklefs, 1976) or that chicks need to accumulate fat early while energy requirement is low to prepare for the increased energy demands during rapid growth (Ricklefs, 1979). Studies in Wilson storm petrels by (Gebczynski & Jadwiszczak, 2000) support the finding that rather than compensating for a poor nutrient diet, parents are over-feeding chicks to counteract periods of extreme weather and unpredictable food supplies. The chicks rely on losing weight near fledging by starvation and dissipation to protect themselves from the increased thermal conductance of obese chicks in cold climates (Gebczynski & Jadwiszczak, 2000). It is also possible that the longer foraging trips are used to protect the parent's body condition rather than the chicks (Baduini & Hyrenbach, 2003). Given the variable volumes of oil present in the wild-type diets, it is likely that identifying the accurate volumes of fat needed in chick diets is heavily biased by the parent birds condition and the external environment influence at the time.

4.7 Future studies

4.7.1 Fortify the finishing diet so it can be fed safely for longer periods.

Future studies into seabird translocations should aim to refine the chick diets towards a safer version to feed for more extended periods, as the diet we have investigated only covers the three to five-week pre-fledging, the ‘finishing’ stage. The importance of chicks transitioning onto a wild diet is still unclear. Return rates improve interpretations of dietary results and need to be accounted for in future translocation plans. Ongoing measurements of success in seabird translocations ideally should span decades rather than years (Jones & Kress, 2012).

4.7.2 Necropsy review

It is highly recommended a comprehensive post mortem review is undertaken of all the chick deaths that have occurred during translocations across multiple species, aiming to identify any deaths that may have a nutritional basis and the incidence of diseases relative to the diets fed. A full necropsy review of translocation deaths was originally part of the aims of this investigation but is beyond the scope of this project. Historic chick deaths with visceral gout, ventriculitis/proventriculitis, cloacal impactions and hepatic lipidosis observed in GFP (McInnes, 2007) along with proventriculitis and visceral gout occurring in this GFP feeding trial, raises concerns there are nutritional imbalances in the diet and possibly issues with food hygiene. Of particular importance for investigating gout is assessing the effect of fluid volumes fed to chicks and salt content, protein, and fat proportions in the diet. While also considering the temperature of burrows, feeding frequency, and the number of handling events that may play a role in disrupting fluid regulation during chick translocations. The exact volume of fluid and fat required is unknown, and it may be that regular provisioning during translocation is disrupting the physiological mechanisms that allow the chicks to survive the long and irregular periods of parental feeding. Research into the water balance and renal physiology in wild, long-strategy foragers is highly recommended.

4.7.3 Fine-tuning diets based on wild diet results

The vitamin and amino acid profiles provided in the supplementary material can be compared with translocation diets for future translocations. Vitamin D3 was not detected in this trial but it is worth considering the importance of Vitamin D2 which may be present in much higher levels.

4.7.4 Future GFP translocations

GFP have proven to be a difficult species to translocate, with a need to refine methods that improve health concerns and fledging parameters to reflect those of naturally raised chicks (Gummer, Taylor, Collen, et al., 2014). Findings from this study are good building blocks for improving dietary oil choices to closely reflect the natural diet, account for weight gain in chicks fed soya oil and identify gout as a risk factor irrespective of the type of oil fed in the diet. Improvements in translocation techniques from GFP will likely extrapolate to other similar-sized burrow nesting, gadfly petrels and shearwaters, that share similar biological traits. Including the Chatham Island tāiko (*P. magentae*) and species that have not yet been translocated, e.g. white-naped petrels (*P. cervicalis*) and white-headed petrels *(P. lessonii*) and potentially surface nesting Kermadec petrel (*P. neglecta*), (Gummer, Taylor, Collen, et al., 2014).

4.8 Conclusion

Artificial diets are pivotal to the success of Procellariiformes chick translocations. There are currently large gaps in knowledge about the basic nutritional requirements of seabird diets, and vast differences are likely to exist between species as they have evolved diverse foraging strategies. A one-size-fits-all artificial diet is highly unlikely to achieve all nutritional requirements, leaving ample room for refinement by improving the similarity of artificial diets to what is known of wild diets, reviewing health concerns in transfers and applying species-specific improvements. The findings from this study comparing the effects of soya oil and fish oil are an important step in understanding how proventricular oil can best be mimicked in artificial diets and the effects that are likely to be observed on health, growth and return rates, ultimately refining chances of translocation success. The problem with reviewing artificial diets in a wild setting is that many confounding factors complicate assessing nutrition directly, so any incremental improvements and results may not be fully understood until chicks return up to a decade later. Measuring RBC fatty acid proportions was an effective tool to assess the impact of dietary lipids on fatty acid retention.

Diet greatly impacted RBC fatty acids even over a brief two-week feeding period during the translocations. Substituting fish oil into the diets improved ratios of functional RBC phospholipid fatty acids at fledging in both long and short term foraging species to levels that more closely resemble wild diet proportions and showed a 5% increase in the return rate in FS. At this level, the return rate of FS was not significantly affected by the artificial diet. Over short feeding periods, a carry-over effect of recently fed wild diets can improve RBC fatty acid proportions to resemble the diets of wild chicks for longer periods than chicks that were not parent-fed recently before transfer. Visceral gout was a concern in long strategy feeders studied, irrespective of the oil fed and requires more investigation and preventative measures to be put in place.

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6 Supplementary Material

A. Sampling method

Figure 48 Sampling method showing placement of gavage tube for proventricular flushing of a grey-faced petrel chick and collection of the samples into a clean container after the proventricular flush or spontaneous regurgitation. Lower right-hand picture shows the collection of a regurgitation sample in a Chatham Island petrel chick

B. Sampling sites

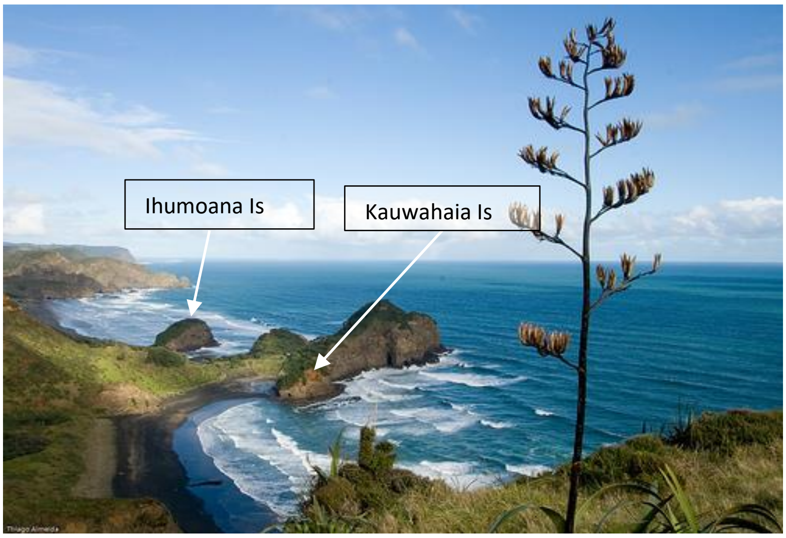

Figure 49 Grey-faced petrel colony at Te Henga showing the two Islands either side of the peninsula Ihumoana Island and Kauwahaia Island, used to compare sample sites within a colony.

C. Amino acids from grey-faced petrels pooled regurgitation and proventricular flushing samples

Table 18. Comparison of amino acids in pooled regurgitation (REG) and proventricular flushing (PVF) samples of three species of burrow nesting petrels (grey-faced petrels, GFP; fluttering shearwaters, FS; and Chatham petrel, CP). Reported on a freeze-dried basis in mg/100mg.

AMINO ACIDS	GFP PVF	GFP REG	FS PVF	*CP (PVF & REG)
Aspartic Acid	3.83	2.19	5.44	3.14
Threonine	1.53	0.89	2.56	1.20
Serine	1.44	0.83	2.25	1.13
Glutamic Acid	4.64	2.74	6.83	3.79
Proline	1.6	0.95	1.93	1.40
Glycine	2.29	1.31	3.03	1.62
Alanine	1.8	1.06	2.95	1.44
Valine	1.83	1.04	3.47	1.48
Methionine	1.72	0.97	1.64	1.36
Isoleucine	1.81	1.03	2.85	1.38
Leucine	2.77	1.6	4.42	2.10
Tyrosine	1.91	1.07	1.93	1.39
Phenylalanine	2.18	1.18	2.47	1.57
Histidine	1.28	0.69	1.74	0.70
Lysine	2.54	1.37	4.17	1.99
Arginine	2.7	1.45	2.79	2.03
Taurine	0.17	0.16	0.36	0.12

*CP samples will likely be an underestimate due to losses during the freeze-drying process.

D. Fatty acid composition of oils in three artificial diets

Table 19. Fatty acid composition of oils in three artificial diets fed during this study compared with the percentage of fatty acids in pooled wild diet proventricular samples from three species of petrel.

Fatty acid analysis.	S/Soy	M/Fish	S/Fish	Grey-faced petrel PVF	Grey-faced petrel REG	Fluttering shearwater PVF	Chatham Petrel PVF & REG
C6:0 Caproic	0.00%	0.00%	0.08%	0.00%	0.00%	0.38%	0.00%
C8:0 Caprylic	0.00%	0.00%	0.00%	0.00%	0.00%	0.25%	0.05%
C10:0 Capric	0.00%	0.11%	0.08%	0.00%	0.68%	1.66%	0.00%
C11:0 Undecanoic acid	0.00%	0.00%	0.00%	0.08%	0.00%	0.00%	0.00%
C12:0 Lauric	0.08%	0.15%	0.11%	0.12%	3.26%	0.25%	0.11%
C13:0 Tridecanoic	0.00%	0.08%	0.11%	0.17%	0.00%	0.13%	0.00%
C14:0 Myristic	5.24%	7.09%	5.54%	3.24%	5.56%	9.43%	5.31%
C14:1n5 - cis-9-Myristoleic	0.08%	0.07%	0.18%	0.17%	0.57%	0.00%	0.27%
C15:1n5 - cis-10-Pentadecenoic	0.00%	0.00%	0.08%	0.00%	0.00%	0.00%	0.00%
C16:0 Palmitic	13.06%	20.40%	19.34%	17.95%	15.74%	41.27%	26.18%
C16:1n7 - cis-9-Palmitoleic	3.95%	9.73%	6.50%	5.35%	6.23%	5.22%	7.46%
C17:0 Margaric	0.16%	0.91%	0.76%	0.83%	0.75%	1.53%	1.02%
C17:1n7 - cis-10-Heptadecenoic	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
C18:0 Stearic	3.15%	4.94%	4.25%	4.73%	3.80%	8.66%	6.12%
C18:1n9t Elaidic	0.00%	0.18%	0.27%	0.23%	0.26%	0.13%	0.70%
C18:1n7t Vaccenic	0.00%	0.08%	0.12%	0.10%	0.09%	0.00%	0.21%
C18:1n9c Oleic	15.00%	17.31%	23.22%	34.28%	33.76%	10.83%	31.12%
C18:1n7c Vaccenic	1.53%	3.17%	3.17%	3.74%	3.47%	2.55%	3.97%
C18:2n6t Linolelaidic	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
C18:2n6c Linoleic	31.21%	4.33%	2.11%	1.27%	1.26%	0.64%	0.21%
C18:3n6 - cis-6,9,12-Gamma linolenic	0.00%	0.22%	0.17%	0.00%	0.00%	0.00%	0.00%
C18:3n3 - cis-9,12,15-Alpha linolenic	4.76%	1.34%	0.99%	0.37%	0.32%	0.38%	0.64%
*C20:0 Arachidic	0.32%	0.33%	0.25%	0.27%	0.24%	0.25%	0.21%
*C20:1n9 - cis-11-Eicosenoic	9.35%	3.63%	9.04%	10.81%	10.58%	5.22%	11.80%
C21:0 Heneicosanoic	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
C20:2n6 - cis-11,14-Eicosadienoic	0.16%	0.23%	0.33%	0.58%	0.49%	0.00%	0.00%
C22:0 Behenic	0.24%	0.19%	0.20%	0.58%	0.20%	0.13%	0.00%
C20:3n6 - cis-8,11,14-Eicosatrienoic	0.00%	0.15%	0.15%	0.00%	0.00%	0.00%	0.00%
C22:1n9 - cis-13-Erucic	0.73%	0.61%	1.15%	1.31%	1.67%	0.64%	1.88%
C20:3n3 - cis-11,14,17-Eicosatrienoic	0.16%	0.27%	0.33%	0.25%	0.28%	0.00%	0.00%
C20:4n6 - cis-5,8,11,14-Arachidonic	0.24%	1.34%	0.97%	0.93%	0.70%	0.25%	0.00%
C23:0 Tricosanoic	0.08%	0.00%	0.08%	0.00%	0.00%	0.00%	0.00%

C22:2n6 - cis-13,16-Docosadienoic	0.00%	0.00%	0.08%	0.00%	0.35%	0.00%	0.00%
C24:0 Lignoceric	0.08%	0.11%	0.08%	0.00%	0.00%	0.38%	0.11%
C20:5n3 - cis-5,8,11,14,17-Epa	5.00%	8.93%	6.74%	3.57%	2.53%	2.55%	0.11%
C24:1n9 - cis-15- Nervonic	0.40%	0.81%	1.08%	1.47%	1.49%	2.68%	2.15%
C22:5n3 - cis-7,10,13,16,19-DPA	0.48%	1.65%	1.37%	0.60%	0.57%	0.25%	0.00%
C22:6n3 - cis-4,7,10,13,16,19-DHA	4.52%	11.64%	11.06%	6.99%	5.16%	4.33%	0.38%

Samples were collected by proventricular flushing (PVF) or from spontaneous regurgitation (REG). All samples were analysed at the Massey University nutrition laboratory. The three artificial diets consist of fatty acids% from S/Soy=Brunswick tinned sardines in soya oil™, M/Fish=Mazuri fish analogue® in fish oil and S/Fish tinned sardines in spring water with fish oil added. The last four columns are pooled samples consisting of two pooled GFP diet samples. Group 1 (n = 8 PVF) and Group 2 (n = 20 REG). FS samples are from 10 pooled PVF from Long Island. CP samples are n = 13 pooled (5 PVF and 8 REG) unfortunately, due to losses during freeze-drying the CP values will underestimate the true value. The artificial diet and GFP samples were analysed from dry matter, while the FS and CP samples are from freeze dried samples.