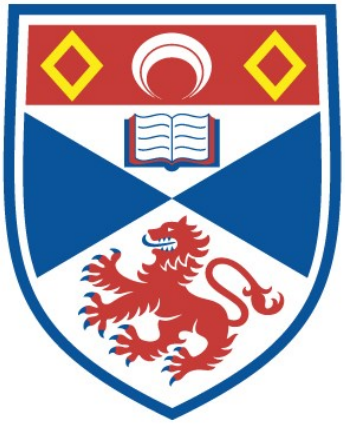
MONITORING ENVIRONMENTAL CHANGE AND ECOSYSTEM HEALTH USING SEABIRD GUANO CHEMISTRY

Elaine M. Tait

A Thesis Submitted for the Degree of MPhil
at the
University of St Andrews



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Monitoring environmental change and ecosystem health using seabird guano chemistry

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University of St Andrews

This thesis is submitted in partial fulfilment for the degree of MPhil

at the

University of St Andrews

1 April 2017

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Abstract

One of the first studies to investigate the use of stable isotopes from seabird guano and extracted uric acid for monitoring environmental changes in diet and trophic relationships of seabirds and anthropogenic pollutant levels, this study supported the hypothesis that guano and uric acid offer an alternative non-invasive sampling technique.

The study found strong evidence of inter- and intra-specific differences in $\delta^{13}C$ and $\delta^{15}N$ signatures across species and breeding locations, which were primarily attributed to the feeding ecology of each species. Patterns of intra-and inter-specific variation were also seen in kittiwakes from the east coast of Scotland where samples were collected within and between years, with considerable isotopic overlap observed in the results suggesting that individuals from these colonies were consuming isotopically similar prey, taken largely from similar regions.

For most species the $\delta^{13}C$ and $\delta^{15}N$ signatures of uric acid were not significantly different from that of guano (<1‰ for $\delta^{13}C$ and 1.2‰ for $\delta^{15}N$) with a highly linear relationship, suggesting that either tissue could be used when studying the short-term (in the order of days) foraging behaviour of seabirds.

When considering the heavy metal burden of seabirds, the present study showed that there are both similarities and differences in trace element concentrations both within and between species that can largely be attributed to dietary variation, although other factors including anthropogenic activities can potentially contribute to this variability in specific locations. With knowledge of the sources and controls on metal variability in diets and bodily accumulation such data derived from seabird guano can provide a potentially useful bio-monitor of trace element concentrations in the wider marine environment.

Stable isotope analysis of seabird guano and uric acid can be used to document changes in diet and trophic relationships that may be associated with environmental change. Using multiple species and sampling locations, such studies can provide an alternative monitoring tool at a range of temporal and spatial scales.

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Chapter 1. Introduction: A review of literature relevant to using stable isotope analysis of guano to investigate the diet and trophic status of Scottish seabirds

Recent declines in seabird numbers and evidence for changes in feeding habitats of some seabird species (Wanless et al. 2005; Mavor et al. 2006; Frederiksen et al. 2004) highlight the need for a better understanding of the interlinked processes that control the functioning and health of marine ecosystems. Furthermore, there is also a clear need to develop monitoring tools that are capable of integrating signals of environmental change and ecosystem health at both the species and broader ecosystem level. As top-level marine predators, seabirds have long been recognised as species that can fulfil this requirement (Cairns, 1987; Furness & Campyshen 1997) with unexpected changes in their numbers, breeding success, diet, trophic status, colony attendance or health providing a strong indication of a food availability or other unknown problem. To date, a mixture of conventional (stomach content analysis, faecal pellets, regurgitations) and more novel (fatty acids, stable isotope) approaches have been used to trace dietary patterns (Quillfeldt et al. 2005), establish trophic relationships (Sydeman et al. 1997; Bocher et al. 2000) and determine pollutant burden (Nisbet et al. 2002) in a range of animals. However, no apparent studies have been undertaken on the stable isotopic analysis of seabird guano to assess its potential to fulfill this role. This review will look at current research in this area, with the aim of drawing conclusions about the utility of stable isotopes, particularly in guano and uric acid, as a viable method for monitoring changes in diet, trophic status and pollutant burden of Scottish seabirds.

1.1 Environmental Change

"I am no longer sceptical. Now I do not have any doubt at all. I think climate change is the major challenge facing the world today" (David Attenborough).

Increasingly governments, conservationists and the general public are concerned about the health and status of the marine environment (Rogers & Greenaway, 2005). The world's oceans and seas have long been under pressure from human exploitation of fossil fuels and from pollution, with many examples of fish stock collapses and subsequent changes in the population ecology of marine species

being a result of excessive human exploitation and environmental changes (Furness & Camphuysen, 1997; Frederiksen et al. 2004). For instance, in the late 1980's, hundreds of thousands of harp seals underwent unusual winter migrations to and along the coast of Norway, while at several seabird colonies in the southwest Barents Sea, numbers of breeding common guillemots collapsed by 70 to 90%. Both these episodes were a response to the collapse of the Barents Sea capelin stock in 1987 due to overfishing (Barrett, 2002).

In addition, the oceans are the ultimate sink in the biogeochemical cycles of many pollutants, and shallow seas have historically been used as a dumping ground for a wide variety of pollutants, including chemicals associated with industries, farms and demoestic houses (Schreiber and Burger, 2001; Arcos et al. 2002, Kojadinovic et al. 2007). Levels of many of these chemicals are elevated in marine and coastal ecosystems because of the influx from rivers, as well as direct pollution (Furness & Rainbow, 1990). With growing human populations, energy demands and industrialisation, the threat from deposition of some substances is increasing (Burger & Gochfeld, 2004). Impacts from increased pollutant loads are already seen in top predators, including marine mammals and seabirds which historically accumulate heavy pollutant burdens (Atwell et al. 1998; Bearhop et al. 2000; Das et al. 2000, Kunito *et al,* 2008; Jepson et al. 2016).

Coastal shelf waters are showing marked and rapid changes due to environmental change, which are predicted to continue into the future. A 1000 km northward shift of warm-water plankton, with a similar retreat of colder-water plankton, has been observed in the north-east Atlantic over the past 50 years as the seas around the UK become warmer (Beaugrand et al. 2003), with further predicted increases in sea temperatures having huge implications for primary production and climate control (MCCIP, 2008). Some of the most immediate effects of recent environmental change, however, are becoming apparent through impacts on biodiversity. The lifecycles of many prey and predators are closely inter-linked, and environmental changes can lead to interdependent pairs of species losing synchronisation (Wanless et al. 2003). In principle at least, this could lead to extinction or changes in the distribution and abundance of species. A study of cetacean distribution around the Scottish coast has shown a northward migration of white-sided dolphins in recent

years, most likely in response to prey abundance (Reid et al. 2005), an observation supported by similar information on basking shark and seal abundances (SCOS, 2005).

1.1.1 Seabirds as indicators of environmental change

Given the economic and environmental value of the marine environment, evaluating its ecosystem status and performance is of high priority. Marine top predators have repeatedly been suggested as indicators of ecosystem health, with the most useful species generally regarded as those that are conspicuous and accessible (Croxall & Prince, 1979; Harris & Wanless, 1990; Montevecchi, 1993; Furness & Camphuysen, 1997; Sydeman et al. 2001). Many seabirds fall within this category and during the breeding season their restricted foraging range associated with colonial breeding means that biological attributes at a given colony can be related to physical and trophic conditions within a defined ocean region (Figure 1.1). Furthermore, the differing life history characteristics of seabird species create a hierarchy in terms of sensitivity to changes in environmental conditions. For example, Furness & Ainsley (1984) predicted that species (i) with energetically expensive foraging methods, (ii) where foraging was restricted close to the colony, (iii) had specialised and inflexible foraging methods, (iv) that had and inability to dive below the sea surface and (v) lacked 'spare times' in their activity budget that could be allocated to foraging if food was scarce; would all be particularly vulnerable to changes in food availability. In contrast, species with large foraging ranges, low foraging costs and considerable periods of time 'off duty' are much more likely to be buffered against reductions in food supply (Wanless et al. 2005). As a result, seabirds offer unique opportunities to study oceanic processes which would otherwise not be possible, and are therefore useful indicators of the condition of the marine environment (Cairns, 1987; Harris & Wanless, 1990; Furness & Camphuysen, 1997; Piatt et al. 2007).

There are a number of particular advantages to using seabirds as monitors of ecosystem health and function. Due to their colonial living, with over 95% of seabirds colonial in nature, seabirds are relatively easy to study and large amounts of data have already been gathered on their ecology and behaviour (Furness et al. 1993; Mallory et al. 2010). Furthermore, since seabirds feed in the upper trophic levels of marine ecosystems, they are highly sensitive to, and show measureable responses

to, a diverse range of factors that can affect the food chain (for example, climatic/oceanographic patterns, commercial fishing pressures, pollution events). Such responses can be rapid and sensitive, with seabirds responding to changes in food supply through such biological parameters as breeding success, attendance behaviour, adult and chick body condition and diet composition; all of which can be observed over a matter of weeks (Wanless et al. 2007). Similarly, pollution events are likely to cause sub-lethal effects to seabirds in terms of embryo or chick development, hatching success or chick behaviour which can be seen over a single breeding season (Kunito et al. 2008; Wing et al. 2014).

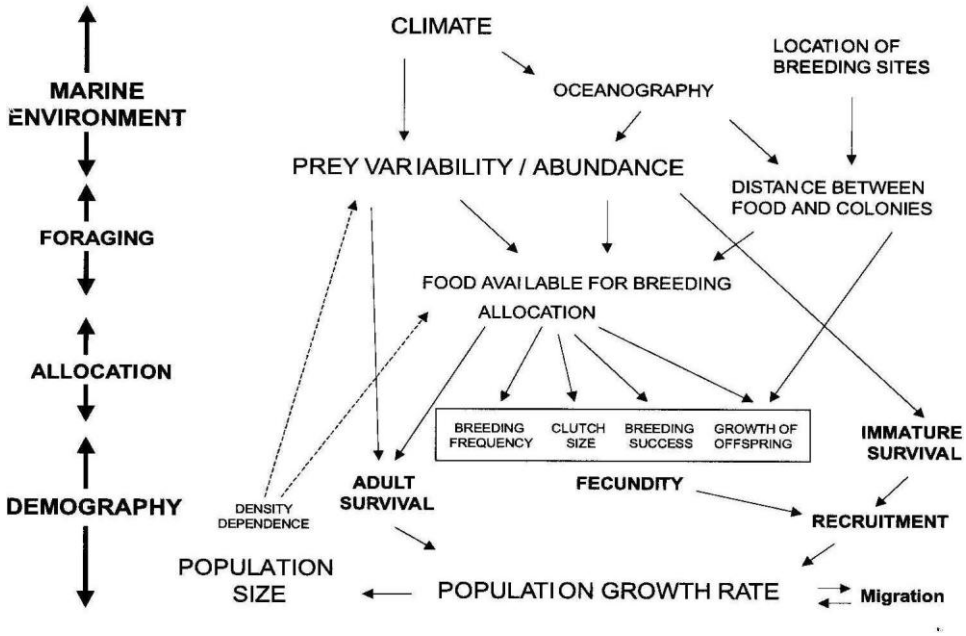


Figure 1.1. Schematic representation of the relationship between demographic traits and the marine environment (Schreiber & Burger 2001)

As highly mobile species, which feed across a broad spectrum of habitats (littoral to pelagic), trophic levels and foraging areas (both inshore and offshore), seabirds offer the opportunity to 'sample' a vast range of habitat niches which is rarely possible with other marine species (Mallory et al., 2010). As a result, by targeting sampling towards a particular species or group of species it is possible to gain information about particular food chains in a chosen geographical region. However, care must be taken when making any inferences within a particular geographical area without a detailed understanding of the life history strategies (e.g., life cycle, moult cycle,

migratory route, prey, foraging range) of the particular species, and often sampling of other bioindicators (including more sedentary species) is often required (Burger & Gochfeld, 2004).

Such is the utility of seabirds, that they are consistently been used as monitors of the condition and health of the ecosystem over local, region and wide-scale geographical areas as well as over time (Walsh, 1990). Indeed, seabirds have been used for many years to monitor heavy metal pollution (Thompson et al. 1998; Becker et al. 2002; Kojadinovic et al. 2007), prey availability (Wanless et al. 2005) and fish stocks and fisheries (Furness & Camphuysen, 1997). Moreover, seabirds have been shown to exhibit responses to changes in marine productivity or climate change (Frederiksen et al. 2006) therefore monitoring of their population, breeding success or survival can provide an indication of conditions across marine regions (Frederiksen et al. 2007). However, to understand how seabirds can be used as indicators of the marine environment, a detailed knowledge of seabird ecology and of the numbers and productivity of many populations, is required (Furness & Camphuysen, 1997).

1.2 Seabird Ecology

Seabirds may be defined as *"birds that spend much of their life at sea, and which are dependent on the marine environment for their food resources"* (Oxford English Dictionary). They are comparatively long-lived, with delayed sexual maturation and breeding, and low annual reproduction rates (Harris et al. 2005). Maximum ages of up to 60 years are documented (*Diomedea epomorpha;* Schreiber & Burger, 2002a) and some birds probably live to a greater age. The corresponding high annual survival rate of well over 90% (Harris et al. 2005, Cairns, 1987) and subsequent low fecundity for many species, are among the reasons that make seabirds ideal for studies of the evolution of life history strategies (Sandvik & Erikstad, 2008). First breeding occurs between 3 and 5 years in most species of seabirds, although it can be as high as 10 to 12 years in some species of Procellariiformes; this is generally assumed to be necessary for long-lived species to attain similar foraging skills to those of adults (Ollason & Dunnet, 1978; Schreiber & Burger, 2001). Most species lay only one to two eggs per clutch and in some cases rearing offspring takes over a

year (e.g., 380 days in wandering albatross, *Diomedea exulans*) resulting in reproduction only occurring every second year. These life history traits are adaptive responses to conditions of living in the marine environment and are generally assumed to be a result of the patchy and unpredictable distribution of marine resources (Rindorf et al. 2000; Wanless et al. 2007).

The life expectancy of seabirds weighs heavily on their reproductive decisions (Charlesworth, 1994). As long-lived species with considerable potential for future reproduction, seabirds favour investment in adult survival over investment in offspring (Cairns, 1987). Studies have shown that small changes in their annual survival rate have unusually large effects on their lifetime reproductive success (Furness & Tasker, 2000; Schreiber & Burger, 2001; Harris et al. 2005) and thus, on the survival of the whole population. That there is considerable variation among seabirds means that the life history of a seabird species may be used as an indication of how vulnerable a species may be in a changing environment (Wanless *et al,* 2005).

1.2.1 Population status, trends and breeding success

Numbers in a population change as a consequence of births, deaths, immigration and emigration (Suryan et al., 2000). During the 20th century many seabird species underwent large increases in population size which were probably linked to changes in fishing practices, either through the availability of large amounts of food in the form of discards and offal (Garthe et al. 1996) or through fishing pressure on large predatory fish leading to increased availability of smaller fish that are the preferred prey of seabirds (Frederiksen et al. 2004). A reduction in human exploitation through the introduction of legislative measures, reduction in discharges of pollution, and eradication of terrestrial predators at vulnerable colonies are also likely to have played a significant role in these increases (Schreiber & Burger, 2001).

Recent years however, have seen some remarkable changes in UK seabird populations. Black-legged kittiwake (*Rissa pterodactyl)* populations in the North Sea have fluctuated widely since 1990 reaching their lowest level for 17 years in 2008 (Newell et al. 2016), probably a result of successive years of poor breeding success due to low availability of their preferred food source (Frederiksen *et al,* 2004 and

2007) coupled with low survival and return rates to colonies (Newell et al. 2013 a, b, c; Newell et al. 2016). Northern fulmars (*Fulmarus glacialis)* have also shown signs of a continued decline since 1996 reaching their lowest level on record in 2012 (Newell et al. 2016), while the number of common guillemots (*Uria aalgae)* on the Isle of May in 2009 was still 39% lower than the previous nine years, following an initial downturn in the population in 2002 (Newell et al. 2013c).

Consistent with these declines has been significant breeding failure across many UK seabird colonies, with some colonies exhibiting considerable breeding failure since 2003 (Frederiksen, 2006; JNCC, 2006), although some species have been showing signs of slight recovery over the last year. Seabirds characteristically have exceptionally high breeding success, with common guillemots fledging on average 0.7 to 0.8 young per pair (Mavor, 2004). However, low fledging success was reported from several colonies in 2003, deteriorating further still through to the 2007 breeding season when only 0.2 young per pairs fledged on the Isle of May (Newell et al. 2013a). Post-fledging survival of kittiwakes in 2007 was also exceptionally low, with many juveniles dying soon after fledging (CEH, *per comms*), while Mavor et al. (2006) documented total breeding failure on Fair Isle in 2005 with most species failing to fledge any young. Such population changes are not limited to the local level, and while populations in various locations have shown different trajectories, decreases are apparent across much larger geographical areas suggesting causes of the declines are associated with large-scale processes.

The evidence suggests that the observed changes in many seabird populations and breeding success in recent years are associated with reduced sandeel availability (Frederiksen et al*.,* 2004 & 2005), yet there is still considerable uncertainty regarding the underlying causing factors. More fundamentally, it is unclear whether these changes are driven by top-down processes or bottom-up influences on food supply. Where there is evidence that bottom-up processes exist, changes in food availability could result from climate driven regime shifts affecting multiple components of the local food webs (Beaugrand et al. 2003; Frederiksen et al. 2004), or as a by-product of fisheries; either directly from discards or indirectly due to fisheries induced changes in prey community structure (Votier et al. 2004b). Furthermore, bottom-up influences can exacerbate top-down processes such as predation, contributing to

population declines. For instance, Oro & Furness (2002) reported that food stress caused by the reduced availability of sandeels around Shetland since the mid 1990's has resulted in heavy predation by Great Skuas (*Catharacta skua)* on black-legged kittiwakes contributing to the already significant population decline of the species.

1.2.2 Diet

Seabirds feed at a range of trophic levels on planktonic and necktonic prey, although the primary food source of most seabirds are densely schooling, small, lipid-rich pelagic fish (e.g., capelin *Mallotus villosus*, Ammodytidae (sandeels), herring *Clupea harengus,* and sprat *Sprattus sprattus*), crustaceans, and cephalopods that occur in the upper- to mid-water column (Hunt *et al,* 1996; Hamer et al. 2007; Wanless et al. 2015). The lesser sandeel, *Ammodytes marinus* is one of the most widely distributed and abundant fish in the North Sea and is an important food source for many seabird species during the breeding season, including auks (Hamer et al. 2007; Newell et al. 2016), terns (Nisbet et al. 2002), kittiwakes (Harris & Wanless, 1997; Lewis et al. 2001; Newll et al. 2013c) and shags (Velando et al. 2005). Harris & Wanless (1997) reported that over a 10 year period, sandeels consistently occurred in 67-100% of regurgitates from breeding kittiwakes on the Isle of May, accounting for 50-98% of the total dietary biomass. These results are consistent with the findings of Velando & Freire (1999) who found that sandeels were represented in 73% of the total number of prey consumed by European shags in Spain, although they did conclude that the number was likely to be considerably higher given the high digestibility of small otoliths. A variety of other prey species are also taken as 'staple foods' (represented in at least 50% of the diet sample), although most seabirds are generally dependent on very few species. ICES (2005) summarized published information on diet composition of seabirds in the North Sea and characterized seabird diet as being composed of sandeels, clupeid fish (herring and sprat), polar cod, a variety of crustaceans, squid and cephalopods in descending order of importance; with prey taken either in the form of discards from fisheries or during more natural feeding.

Seasonal variation in diet

Seabird diet composition often varies within and between years, especially with respect to the proportion of schooling fish in the diet (Rindorf et al. 2000; Lewis et al. 2001). Many fish species are known to show rather different distributions and

patterns of activity during the course of a year and, coupled with constantly changing energy demands associated with courtship, incubation, raising chicks, fledging young and winter survival; these factors will contribute to these inherent changes in preference amongst bird populations (Furness, 2002). Building on previous studies by Wanless & Harris (1997), Lewis *et al* (2001) documented a well-defined seasonal change in the diet of kittiwakes in the North Sea from a predominance of planktonic crustacea in early spring (April), to adult (1+ group) sandeels in April and May, to the youngest of the age group (0 group) sandeels in June and July, supporting predictions that age-specific, seasonal changes in sandeel behaviour will affect a seabird's diet; while Coulson & Thomas (1985) emphasised the importance of clupeids (approximately 75%) and gadoids (10%) for pre-breeding kittiwakes from NE England in February and March. Similar dietary patterns were also documented in European shags on the Galician coast (NW Spain), changing from predominantly gobies and smelts in February and March to sandeels as the dominant prey in the spring (Velando & Freire, 1999). Comparative studies in north east Scotland found no similar well-defined seasonal variation in the diet of shags (JNCC, 2004).

Despite the wealth of information on the diet of seabirds at colonies, there is still a lack of sufficient information on diet away from the coastal colonies and during the winter months (Barrett et al. 2007). Blake et al. (1984) suggested that winter diets of seabirds generally show a wider variety of prey species than in summer, although Wanless et al. (1993) noted that caution should be taken when interpreting these assumptions as some adults are known to self-feed on a wide spectrum of prey away from the colony often going unnoticed in studies based on regurgitation in the colony. Ouwehand et al. (2004) documented clupeids, gadoids and sandeels as important prey species for both guillemots and razorbills wintering in south-eastern North Sea, supporting earlier suggested that clupeids and gadoids are key species in the North Sea for winter survival of auks (Harris & Bailey, 1992; Camphuysen, 1996); while Blake et al. (1984) highlighted the relative importance of these prey for guillemots in the post and pre-breeding seasons. Such findings however, are not consistent with studies in Shetland where Ewins (1990) reported that invertebrates assumed increased importance in the diet of black guillemots, *Cepphus grille,* during the winter months, although the authours did suggest that the findings were likely to be a relfection of reduced availability of their predominant prey. Increasingly

Seabird Species	Diet	References
Northern Gannet, *Morus bassanus*	Wide variety of prey taken, although mackerel (*Scomber scombrus)*, herring *Clupea harengus)* and sandeels (*Ammodytes marinus*) form the main components of the diet. Exhibit annual changes in the most abundant prey item dependent on fluctuations in prey availability (e.g., mackerel the most common prey in 1998 for gannets from the Bass Rock, while in 2001 the diet comprised similar proportions of mackerel and sandeel).	Garthe *el al,* 2005 IMPRESS Report, 2004; Hamer *et al,* 2001
Black-legged kittiwake, *Rissa tridactyla*	High dependence on the lesser sandeel, *Ammodytes marinus.* Well-defined seasonal shift from 1+ group sandeels in April and May, to 0 group sandeels in June and July, although there is evidence for an increasing frequency of pipefish in the late summer diet (July/August) at many colonies. In the North Sea, planktonic crustacea form an important component of the diet in early spring (April).	Wanless & Harris, 1997 Lewis *et al,* 2000 Daunt *et al,* 2002
European shag, *Phalacrocorax aristotelis*	Almost completely dependent on sandeels with 1+ age group forming the core diet. 0 group sandeels becoming increasingly frequent in regurgitate samples. Other prey items taken include gadoids and clupeids. Gadoids are particularly important to shags during the breeding season on the west coast of Scotland.	Wanless *et al,* 1991 IMPRESS Report, 2004; Isle of Canna Report 2007
Common guillemot, *Uria aalgae*	The main prey items of guillemots are clupeids (mainly *sprattus sprattus*), the lesser sandeel (*Ammodytes marinus*) and gadoids (whiting *Merlangius merlangus).* Clupeids are becoming increasingly important in provisioning chicks in recent years, while at some colonies the percentage of sandeels is decreasing. Clupeids and gadoids are important for winter survival of guillemots.	Blake *et al,* 1985 Ouwehand *et al,* 2004
Razorbill *Alca torda*	The main prey of razorbills is 0 group sandeels (*Ammodytes marinus)* and clupeids (mainly *sprattus sprattus*). Clupeids and gadoids are important for winter survival of razorbills in the North Sea.	Blake *et al,* 1985 Ouwehand *et al,* 2004
Atlantic puffin *Fratercula arctica*	The main prey items of puffins are clupeids *(*mainly *sprattus sprattus*, sandeels and rockling.	Isle of May Report 2006, 2007 Harris *et al,* 2005
Northern fulmar, *Fulmarus glacialis*	The diet of the Northern fulmars is varied, especially annually and regionally. They show high dependence on fishery based waste (offal) and discards, which form the main component of the diet in most regions. Crustacean, herring, small squid and jellyfish are also important food sources, although there relative importance varies markedly between years.	Thompson, 2006 Schreiber & Burger, 2001 Garthe *el al,* 2005
Herring gull *Larus argentatus*	The herring gull is an opportunist and scavenger that feeds on discarded fish offal, chicks, mammals, eggs, invertebrates and carrion	Isle of May Report 2006, 2007
Great skua *Catharacta skua*	Highly variable between locations. Generally consists of: other seabirds (petrels, kittiwakes, auks), discarded gadoids and sandeels.	Monaghan, 1992

Table 1.1. Diet composition of the main seabird species in the British Isles

however, seabirds in the North Atlantic have been shown to make extensive use of discards during the winter months, with the distribution patterns of kittiwakes and fulmars closely correlated with fishing vessel activity, highlighting the importance of this food source for winter survival (Mavor, 2004; Kubetzki et al. 2009; Frederiksen et al. 2011; Quinn, 2014).

Annual Variation in diet

Annual changes in diet from one prey species to another are usually seen as a response to annual fluctuations in prey availability or strong environmental conditions (Camphuysen, 2004). Studies on gannets from Bass Rock in 2002 and 2003 documented 0-group sandeels as the most abundant prey item in terms of both frequency of occurrence and biomass, occurring in 75% of regurgitations (Camphuysen, 2004). However, in periods of low sandeel availability a greater proportion of the diet comprised of other species, with mackerel the most common prey in 1998 (Hamer et al. 2000) while in 2001 the diet comprised similar proportions of mackerel and sandeel (Lewis et al. 2001). Similar patterns can also be seen in guillemots on the Isle of May where long-term monitoring of prey brought back to the colony has shown that the importance of older (1+ group) sandeels have been declining in recent years in favour of an increasing trends towards clupeids (e.g. sprats), although 0 group sandeels still occurred frequently in the diet (Newell et al. 2013c; Newell et al. 2016).

Diet switching

The ability of an animal to switch from its primary prey to an alternative food source, particularly during times of low prey abundance (Suryan et al. 2000) is very much a species-specific response. The ability of surface-feeding birds such as terns and kittiwakes to switch prey during the chick rearing period can be restricted at some colonies because alternative prey are often unavailable. Limited by both surface-shoaling fish and the need to obtain energy-rich food for chick development, these species have a fairly specialized diet during the breeding season. In fact, Hamer et al. (1993) documented that kittiwakes in the North Atlantic did not change their diet when their principal prey declined, but instead increased their overall foraging effort to obtain sandeels. This observation, however, has not been supported by findings from the east coast of Scotland where snake pipefish were consistently recorded in the diets of kittiwakes during the 2007 to 2009 breeding seasons (Newell et al.

2013a, b, c). Whether this is a response to an increasing abundance of snake pipefish, most likely due to the influx of warmer waters, or an absence or redistribution of sandeels in the water column (Caradden et al. 2002) is not currently known. In contrast, great skuas, *Catharacta skua,* and Northern Gannets in Shetland have shown much more diverse foraging strategies. Following the crash of the sandeel fishery in 1979, the diet of the great skua shifted from sandeels to whitefish discards, and more recently to seabird predation (Votier et al. 2004 & 2007), while gannets showed a predominance of herring and mackerel in their diet (Monaghan, 1992; Votier et al, 2011; Quinn, 2014).

1.2.3 Distribution and foraging ecology

A major constraint on breeding seabirds is the distance between the colony and their feeding grounds (Weimerskirch and Cherel, 1998; Thaxter et al. 2012), with foraging distance limited by the need to return repeatedly to the nest site to meet the chick's nutritional requirements, as well as sustaining their own (Daunt et al. 2002; Durant et al. 2003). Resource availability within the foraging range of a species should therefore be crucial to its overall success (Pinaud & Weimerskirch, 2002). Gaining an insight into the distribution of seabirds whilst at sea is particularly difficult because of the huge logistic and financial constraints involved in working in the marine environment (Camphuysen, 2004). Previously, most information on the distribution and foraging behaviour of seabirds has been obtained from ship- or land-based observations during the breeding season. However, the increased use of locational transmitters and minaturised activity loggers (e.g., Hamer et al. 2000; Evans, 2012; Soanes et al. 2013) has greatly improved understanding of the foraging ecology, strategies and distribution of many seabird species (Garthe et al. 2000; Daunt et al. 2002).

Foraging behaviour

It is assumed that the way seabirds distribute themselves with respect to the environment depends largely on the distribution, abundance and predictability of prey (Bell, 1991; Camphuysen, 2011). However, the weak links between seabird distribution patterns and prey abundance at the smaller scales seems to support findings that individual seabirds tend to specialise on certain habitat characteristics and environmental conditions rather than responding to the constantly changing

distribution of the highest concentrations of prey (Schreiber & Burger, 2001;). Recent studies have shown that top predators, including seabirds, respond to physical oceanic gradients where productivity is enhanced or where there are increased abundances of fish, larvae and zooplankton (Franks, 1992). Camphuysen et al. (2006) emphasised the importance of shallow sea fronts for foraging seabirds in the North Sea, with the greatest densities of kittiwakes occurring in response to constantly changing hydrographical conditions, which often acted as an 'invisible' barrier for foraging seabirds. Usually the influence of oceanography on seabird distribution is through variations in surface salinity, transparency and thermal stratification. For example, Garthe (1997) observed that for fulmars, and to a certain extent guillemots, occurrence was correlated with highly saline, thermally stratified water; while Ribic & Ainley (1997) found that Sooty Terns and Wedge-tailed shearwaters (*Oceanodroma leucorhoa*) were associated with deep thermoclines and low salinities. Other species, such as European shags, respond to distinct benthic characteristics and as a consequence are found foraging in near-shore waters, although they are occasionally found within estuaries (Wanless et al. 1999; Isle of May, 2005).

The foraging behaviour of seabirds varies in response to a range of factors including tidal cycle (Irons, 1998), time of day (Becker et al. 1993), photoperiod (Williams et al. 1992) and sea currents (Montevecchi & Myers, 1995). Black-legged kittiwakes (*Rissa tridactyla*) in Alaska have been shown to shift their foraging schedule to coincide with daily tidal cycles, with some individuals consistently foraging on a flood tide while others track the diurnal ebb tide (Irons, 1998). Breeding European shags exhibit clear diurnal patterns in their dives, averaging between 8.5 and 34.6m (Wanless et al. 1999). A similar pattern is evident among genatoo penguins, averaging 40m at dusk and 80-90m at midday. This pattern likely reflects the vertical migration of prey at various times of day, but could also relate to differences in the attenuation of light with water depth (Wanless et al. 2000; Garthe et al. 2000). Water clarity also may exert selective pressure on seabird foraging modes and has been shown to constrain the foraging of gannets and terns during periods of low water clarity (Hamer et al. 2001). Most seabirds are visual predators and rely on sight to forage most actively during the day (Hamer et al. 2001). Some species, however, regularly forage at night (e.g., mainly procellariiformes) using smell to detect food

(Nevitt et al. 1995), although this is likely to be a response to the distribution of prey as opposed to an active choice.

Most individuals exhibit preferences for particular sites and return to them frequently suggesting that they rely more on 'local knowledge' or memory than information transfer to locate prey patches. Daunt et al. (2002) found that individual black-legged kittiwakes in the North Sea consistently travelled to feeding grounds on a bearing of between 45^{o}N and 135^{o}N to a distance of approximately 73 ±9 km from the colony, which the author suggested was a response to the Shallow Sea front and subsequent distribution of sandeels at Marr Bank and Wee Bankie. Similar patterns were also found amongst gannets from Bass Rock where individuals were found to consistently forage in a single direction, or at most two directions, with very similar bearings on successive trips (Quinn, 2014). Not all individuals however adopt similar behaviour and, the extent of area fidelity is likely to vary by species, season and region. Wandering Albatrosses, *Diomedea exulans*, which forage over hundreds of kilometres, sometimes use the same foraging area on consecutive trips but also explore new areas during the same trips (Weimerskirch et al. 1993). Similarly, Wanless et al. (1990, 1991) showed that individual guillemots and razorbills foraged in similar areas on some consecutive days but do not exhibit strong area fidelity. In contrast, individual shags foraged over a range of areas, possibly because their benthic prey, although predictable, can become depleted (Birt et al. 1987).

Foraging range

Most studies on the foraging patterns of seabirds have been conducted during the breeding season when birds must periodically return to the nest site to incubate eggs or to tend to young (Durant et al. 2003). As a result, it is generally assumed that foraging ranges of seabirds are constrained during the breeding season (Weimerskirch & Cherel, 1998). Nonetheless, some species continue to range widely, with the most noticeable being several species of Procellariiformes that have been recorded travelling over 15,000 km of ocean during a single foraging trip (Durant et al. 2003). The duration of foraging trips are variable between species, but in the Northern Hemisphere are generally thought to last between 12 and 24 hours (Camphuysen, 2011), although trips of 5 days or longer may occur (*Fulmarus* sp).

	Northern Gannet, *Morus bassanus*	**Black-legged kittiwake,** *Rissa tridactyla*	**European shag,** *Phalacrocorax aristotelis*	**Common guillemot,** *Uria aalgae*	**Northern fulmar,** *Fulmarus glacialis*
Foraging strategy	Plunge-diving or scoping strategy (scoping generally associated with MSFA's)	Dipping or shallow plunge diving (usually in association with foraging guillemots)	Foot-propelled pursuit diving	Wing propelled pursuit diving from the water surface.	Dipping or shallow plunge diving
Foraging range (from colony)	Highly variable foraging range from <50 to 540 km. Max foraging range at: Bass Rock – 540 km (Hamer *et al,* 2001); Great Saltee – 240 (Hamer *et al,* 2001); Shetland – 129 km (Garthe *et al,* 1999).	Foraging range from <1km to 160 km dependent on feeding conditions, although generally between 14 and 84 km from colony (Pollock *et al,* 2000). Maximum foraging range from the Isle of May is 73 +/_ 9 km (Daunt *et al,* 2002)	Coastal species, rarely forage more than 10 km from the breeding colony (Wanless *et al,* 1991)	Highest densities are generally found within 25 km of the colony, although foraging ranges can be as far as 110 km (Kincardine & Deeside) (References: IMPRESS Report, 2004; Pollock *et al,* 2000)	Generally forage offshore, with foraging ranges in excess of 80 km.
Foraging depth	Foraging depth is variable between sexes (females - 4 m; males - 2m). Dive depth also variable with tidal fronts.	Surface feeders. Usually feed within 1m of the surface.	Benthic feeders with foraging depths between 20 and 27m (Wanless *et al,* 1999).	Maximum foraging depth between 60 and 70m, with average depth in the region of 39-43m dependent on foraging conditions.	Surface feeders. Usually feed within 1m of the surface, predominantly on discards.
Flock formation / feeding associations	Mainly feed in association with MSFA's., with most birds found in association with fishing vessels.	Mainly feed in association with natural MSFA's, although small numbers feed either solitary or with conspecifics.	Coastal feeders. Do not form MSFA's.	Majority of foraging birds feed in natural MSFA's, and the rest either solitary or within conspecifics.	Majority found in association with fishing vessels.
Habitat Characteristics	Utilise a wide range of habitat characteristics, but show a preference for well mixed waters.	Tend to feed in slightly stratified waters.	Found exclusively in freshwater influenced coastal waters, with distribution tied closed to sandy habitats.	Utilise the widest range of habitat characteristics.	Utilise a wide range of habitat characteristics.
Winter distribution	Disperse from the colony westward into the North Atlantic and southwards to African waters. Exhibit relatively high densities in UK waters in February and March. (Tasker *et al,* 1990)	Widespread distribution. Birds generally disperse to the Bay of Biscay, the North Sea and westward to the North Atlantic (Lloyd *et al,* 1991)	Remain in inshore, coastal waters year round.	Birds generally disperse into the North Sea and North Atlantic. Some individuals are known to make regular trips to the breeding ledges in January and February (Blake *et al,* 1984)	Widespread distribution. Individuals regularly sighted around the UK coast during the winter months.

Table 1.2. Foraging strategies and characteristics of select seabird species found around the UK coast

Foraging ranges of seabirds are generally classified as coastal, inshore, offshore and pelagic. Coastal feeding species such as shags and cormorants usually forage in waters less than 40m deep and within 10km from their breeding colonies, although they are sometimes known to venture into estuarine habitats (Wanless et al. 1991). Inshore species such as the auks and terns are typically highly concentrated in near-shore waters (e.g., guillemots usually within 25km of the colony and puffins within 40km), but with lower densities further offshore (Malvor *et al.*, 2000). Offshore and pelagic species such as Manx shearwaters, gannets and fulmars, range more widely (e.g., maximum ranges of >300, 590 and 580 km respectively) dependent on foraging conditions (Thaxter et al. 2012) while Pinaud & Weimerskirch (2002) documented a foraging range of 200-300km for black-browed albatross in Kerguelen during incubation. Other species alternate or mix long (pelagic) and short (inshore) foraging trips during incubation (Weimerskirch et al. 1994). Stable isotopic studies of adults returning to the colony suggest that parents use short trips almost exclusively to gather food for chicks, whereas long trips are a strategy to replenish energy lost during chick-provisioning trips. Foraging ranges can also be highly variable within families and between individuals (Wanless et al. 1990).

The foraging ranges of seabirds are governed by a combination of factors including foraging strategy (Camphuysen, 2006), prey availability (Wanless *et al.,* 2005) and mode of flight (Durant et al. 2003), although Jovani et al. (2016) recently found a interspecfic correlation between colony size and foraging range. The use of underwater pursuit diving strategies by auks has resulted in reduced flight efficiency and high energy costs (razorbills require 64% more energy to fly than petrels), subsequently limiting their foraging distribution to near-shore waters close to the colony. Others species such as fulmars and gannets which exhibit more energy efficient gliding flight can range much more widely (Thaxter et al. 2012), although calm weather in summer has been associated with wrecks (e.g., mass morality events) of fulmars (Camphuysen, 1989a) probably a result of increased energy costs associated with flapping flight in birds which are adapted to gliding in the wind (Furness & Bryant, 1996). Some studies have observed seabirds actively adapting their foraging range to changing environmental conditions. For instance, Furness (1996) reported seabirds foraging further away from the colony during adverse

environmental condition, although such responses have been shown to have a knock on effects in terms of colony attendance and chick survival (Wanless et al. 2005).

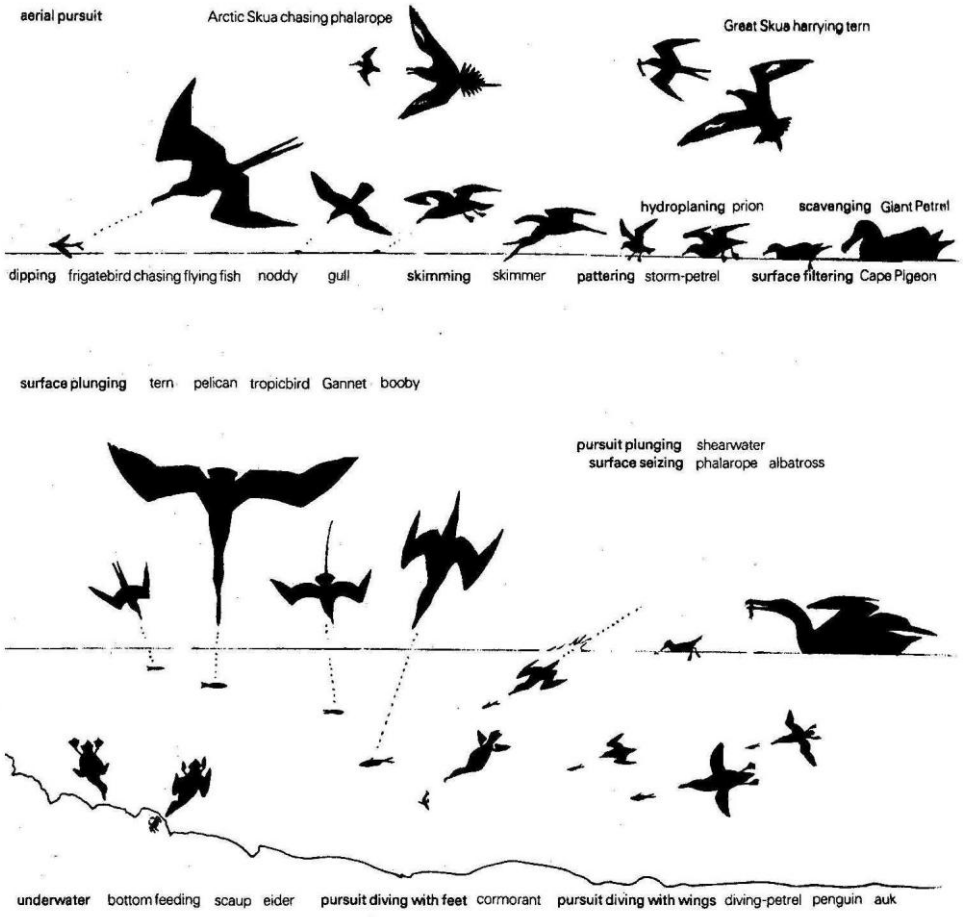


Figure 1.2. Various feeding methods used by seabirds in obtaining prey (from Schreiber & Burger, 2001).

Foraging strategies

The constraints imposed by a largely sparse, patchy and often unpredictable food resource (Ashmole, 1971; Hamer, 2001; Rindorf, Wanless & Harris, 2000) means that seabirds exploit these resources through the selection of highly variable and often species-specific foraging strategies which in turn determine how they respond to changes in food availability or environmental conditions (Daunt et al. 2001). Ashmole (1971) identified six categories of foraging techniques used by seabirds: *wing propelled underwater swimmers* (guillemots, razorbills); *foot-propelled*

underwater swimmers (shags, cormorants); *plunge-divers* (gannets, terns); *surface feeders* (kittiwakes, fulmars); *kleptoparasitism* or piracy (great skuas); and *capturing prey while flying at the sea surface* (skimmers) (Figure 1.2).

Seabirds generally use each of these methods to different degrees, and many species are known to make use of several methods (Schreiber & Burger, 2001). Camphuysen (2011), for example, reported that northern gannets tended to adopt deep or shallow plunge diving strategies when feeding alone, but actively switched to a scooping strategy when feeding in association with multi-species assemblages, most likely a consequence of increased availability of prey in the surface waters. Conversely, some species are highly specialised, adopting single foraging techniques to access prey (Furness & Tasker, 2000). Water clarity may also exert selective pressure on seabird foraging modes and has been shown to influence the foraging and distribution of seabirds (Ainley, 1977; Camphuysen, 2011) with plunge diving strategies more common in clear waters and pursuit diving positively correlated with increasingly turbid waters. Such responses most likely reflect plunge divers requiring relatively clear waters to keep a visual fix on their prey, while pursuit divers rely more on surprise to catch prey (Wanless & Harris, 1997).

1.2.4 Analytical approaches for monitoring the diet and trophic status of seabirds

Analysing the diet and trophic position of a top-level marine predator is important to the understanding of marine ecology food webs (Ruiz-Cooley, 2004). In recent years, attention has focused on the possible role of climate change on sandeel distribution and abundance in the declines of specific seabird species around the UK coast (Montevecchi & Myers, 1997; Durant et al. 2003; Sandvik et al. 2005, Wanless et al. 2007). Evaluating hypotheses such as these requires specific information on the diet of seabirds in the areas. However, the application of traditional methods (i.e., regurgitates, faeces, stomach contents, observations) for studying diet or foraging behaviour of animals has known biases and limitations (Burns et al. 1997; Kelly, 2000; Hamond & Wilson, 2016; Wilson & Hammond, 2016). Observational data, particularly for seabirds, are typically limited to breeding colonies during spring and summer months and reveal little about the foraging habits of these animals at other

times of year (Dunnet et al. 1990). Moreover, often the assumption is made that nestling and adult diets are similar, but studies have shown that adults feed on prey of different sizes and species than they deliver to their chicks (Wanless et al. 1993; Shealer 1998) and that their foraging behaviour differs between self-feeding and chick provisioning (Forero & Hobson, 2003).

The analysis of stomach contents and regurgitates/faecal pellets can also provide useful information about the diets of many seabirds, but are often biased in favour of the remains of durable hard parts due to differential rates of digestion (Hobson and Clark, 1992; Lajtha and Michner, 1994; Hilton et a. 2000b; Phillips *et al.,* 2005). For example, Votier et al. (2001) reported an overestimation of the proportion of birds in the diet of great skuas *Catharacta skua* when using pellet contents. Hobson & Welch (1992) found that seabirds in Barrow Strait and Lancaster Sound consumed more lower trophic level invertebrates than previously suggested through conventional dietary analysis highlighting the importance of more systematic, controlled studies to calibrate sampling techniques (Brown & Ewin, 1996). Furthermore, the use of temporal dietary information is often difficult to quantify, leading Dalerum et al. (2005) to argue that many of these methods provide only a “snapshot” of the diet at a point in time, and may not be representative of the typical long-term diet of the animal.

To avoid some of these difficulties, researchers have made inferences about diet in marine mammals and seabirds based on diving behaviour, foraging location and the behaviour of potential prey (Wanless et al. 1999; Harris et al. 2005). However, observed errors and biases with both these and conventional dietary analysis have created a need to employ additional dietary methods that may more accurately reflect the long term diet and that can be obtained from sampling free-living animals less intrusively (Herman et al. 2005). Analysis of stable isotope ratios has recently emerged as a powerful technique and has been increasingly used to assess the dietary preferences and trophic position of a diversity of seabirds and marine mammals (Forero et al. 2002; Weimerskirch et al. 2005; Bearhop, 2006; Bird et al. 2008; Bond & Jones, 2009).

1.3 Stable Isotope Analysis

You are what you eat …… or are you? Natural variation in stable isotope ratios of elements has long been a tool used by geochemists and palaeo-oceanographers (Lajtha & Michner, 1994). Since the landmark publications of DeNiro & Epstein (1978, 1981), their application in ecological and environmental studies has been widespread with stable isotopes increasingly used to improve our understanding about dietary patterns of animals (Gannes et al. 1997 & 1998; Thompson et al. 1995). Stable isotopes have also been utilised to discriminate between animals living in different biomes (Furness et al. 2006, Inger et al. 2006), to trace the migration patterns of birds (Forero & Hobson, 2003), to unravel trophic relationships in food webs (Hobson & Montevecchi, 1991, Sydeman et al. 1997) and more recently to understand the behaviour of contaminants since isotopic measures may allow insights into dietary sources and trophic levels of individuals (Elliott & Scheuhammer, 1997; Das et al. 2003; Krahn et al. 2007). The utility of stable isotopes in such ecological studies is based on the following principles. First, the isotopic composition of a consumer reflects that of its diet in a predictable manner (DeNiro & Epstein, 1978; Hobson & Cark, 1992a). Second, because tissues of a consumer turnover at different rates, they integrate information on diet over different temporal scales (Tieszen et al. 1983). Third, the isotopic signatures of potential dietary sources are often isotopically distinct and hence are distinguishable from one another (Gannes et al. 1997).

1.3.1 Stable Isotopes and their abundance in nature

Stable isotopes are naturally occurring stable forms of elements with differing nuclear masses that cause each isotope of the same element to behave slightly differently in biogeochemical processes (Schoeller, 1999; Rubenstein & Hobson, 2004). Traditionally the use of stable isotopes in animal ecology have focused on the ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$), since the isotopic composition of these elements varies in relation to the diet, trophic level and foraging location of a consumer (Owens, 1987). DeNiro & Epstein (1978, 1981) were among the first to observe that the carbon and nitrogen isotope values change between organisms and their diet through the process of 'fractionation.' During fractionation light and heavy isotopes pass through the body at different rates due to biochemical processes,

resulting in the heavier isotopes being selectively retained within the body while the lighter isotopes are preferentially released (DeNiro & Epstein, 1978; Tieszen et al. 1983; Peterson & Fry, 1987; Sponheimer et al. 2003). As a result, animals generally become enriched in the heavier isotopes and therefore exhibit higher values than their diet (DeNiro & Epstein, 1978; Tieszen et al. 1983; Peterson & Fry, 1987), emphasising the commonly translated phrase. '*you are what you eat...plus a few per mil"* (Voigt & Matt, 2004).

Yet, while the underlying physiological, biochemical and biophysical processes that drive this observed response are not fully understood (these will be discussed later), the degree of enrichment of an animal over its diet has been extensively studied (DeNiro & Epstein, 1978; Lajtha & Michner, 1994; Kelly, 2000; Herrera & Reyna, 2007). Following the earlier studies of DeNiro & Epstein (1978), which were among the first to provide evidence that the isotopic composition of a consumer reflects that of its diet in a predictable manner; the general view of current literature is that marine food webs show a step-wise enrichment of approximately 3.0‰ to 5.0‰ in nitrogen (due to preferential loss of ^{14}N during protein synthesis) and 0.8‰ in carbon with each trophic level, although important variation may exist among tissues due to differential isotopic routing (Hobson et al. 1994; Quillfeldt et al. 2005; Martins et al. 2012).

1.3.2 Use of stable isotopes in understanding trophic relationships

Carbon

The (nearly) conservative transfer of carbon to a consumer from its diet means that stable isotope analysis is an ideal tool for tracing sources of primary productivity in food webs (Schreiber & Burger, 2001). Carbon sources enter the base of food webs with characteristic signatures as a consequence of differential processes of carbon fixation during photosynthesis, resulting in foods derived from marine sources generally showing different, and often more enriched, isotopic compositions than those from terrestrial and freshwater sources (Chisholm et al. 1982). The existence of these differences, and their preservation throughout food webs, have resulted in carbon stable isotope ratios being successfully used to both identify the relative

contributions to an animal's diet of foods from different origins (Hobson, 1986) and to distinguish between plants exhibiting different photosynthetic pathways (DeNiro & Epstein, 1978). For instance, the difference in isotopic composition between C3 and C4 grasses have been used to discriminate grazers from browsers in African elephants (DeNiro & Epstein, 1978), whilst using stable carbon isotope analyses of bone collagen of gulls, Hobson (1986) was able to estimate the relative proportions of marine and terrestrial protein in the diet of gulls.

Stable carbon isotopes have also been used successfully on a smaller spatial scale to determine foraging locations of animals (Hobson et al. 1994; Herman et al. 2000; Martins et al. 2012). Within the marine environment, $\delta^{13}C$ signatures are commonly used to differentiate between biota utilising inshore/benthic versus pelagic food chains since benthic and pelagic biomass are known to have different isotopic signatures (France 1995, Hobson et al. 1994; Herman et al. 2000). Using carbon isotope signatures, Becker et al. (2000) was able to highlight spatial segregation between two species of diving petrels in South Georgia with *Pelecanoides urinatrix* foraging in coastal waters in the close vicinity of the colony and *P. georgicus* in more offshore waters; while similar work by Hobson (1995), Thompson et al. (1996) and Sydeman et al. (1997) identified characteristic isotopic differences in benthic/pelagic, and inshore/offshore feeding, and in a range of seabird species (Figure 1.3). Segregation on the basis of latitudinal gradient (Bocher et al. 2000) and fronts (Quillfeldt et al. 2005) have also been extensively studied.

Nitrogen

Differences in nitrogen isotope ratios are typically used to determine trophic level, and in cases where the isotopic ratios of different prey are known, actual diet (Hobson., 1993; Hobson et al. 1994; Sydeman et al. 1997; Perkins et al. 2014). Many of the earlier applications of the stable isotope approach in animal ecology have primarily focused on trophic relationships in communities, which have been instrumental in establishing important and novel insights into intra- and inter-species trophic relationships and defining trophic interactions on both spatial and temporal scales (Hobson & Montevecchi, 1991; Hobson et al. 1994; Thompson et al. 1995; Thompson et al. 1999). In the Gulf of Farallones, California, stable isotope analysis

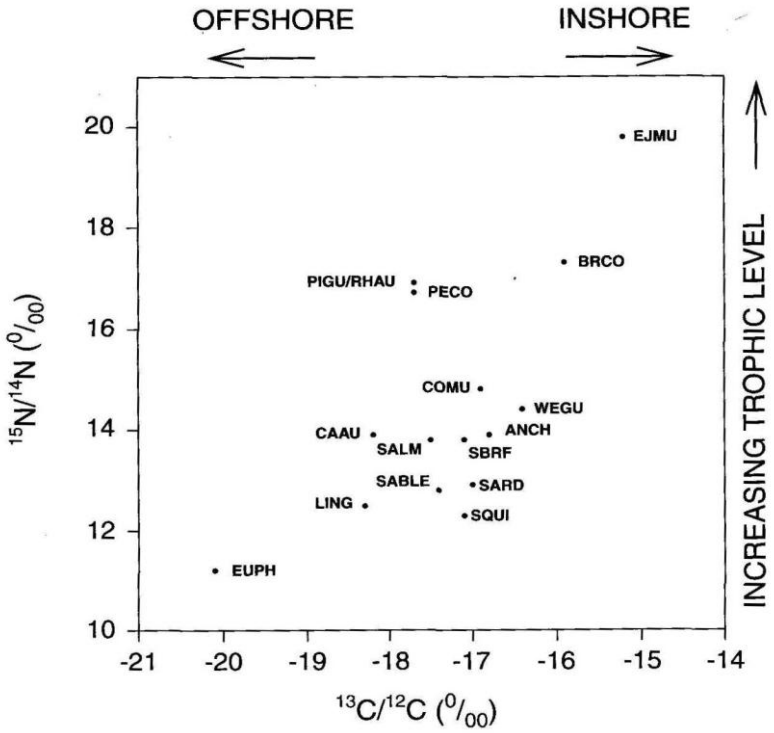


Figure 1.3 Trophic structure in a range of seabird and marine species in the Gulf of Farallones. Abbreviations: EUPH (krill – euphausiids), LING (lingcod), SQUI (squid), SARD (sardine), SABLE (sablefish), SALM (salmon), SBRF (short-bellied rockfish), ANCH (anchovy), CAAU (Cassin's Auklet), WEGU (Western Gull), COMU (Common Murre), PIGU (Pigeon Guillemot), RHAU (Rhinoceros Auklet), PECO (Pelagic Cormorant), BRCO (Brandt's Coromorant), EJMU (northern sea lion). Taken from Sydeman *et al.,* 1997.

was used to infer relative trophic relationship of several seabird species during the breeding season (Sydeman et al. 1997), answering important questions on spatial segregation between those species adopting piscivorous (high ^{15}N signatures) and planktivorous (lower ^{15}N signatures) feeding habitats (Figure 1.3). Similar results were also observed by Thompson et al. (1999) who documented strong patterns of higher trophic level and offshore feeding in birds from North Iceland (in comparison to South Iceland) indicative of a diet dominated by lipid-rich capelin. Temporal dietary shifts have also been well documented. Using stable isotope analysis of feathers, Thompson et al. (1995) reported long-term declines in the trophic status of the Northern fulmar at two North Atlantic colonies, most likely a consequence of a

changing food resource (cessation of whaling and availability of offal); while Thompson & Furness (1995) observed strong seasonal trophic shifts (up to 3‰) between winter and summer in the diets of Northern fulmars.

In more recent years, however, there has been a clear evolution from these largely broad scale approaches to more individual based analysis (Forero & Hobson, 2003). The identification of individual characteristics that determine differences in diet and feeding ecology within populations could be of importance to understanding population dynamics (Forero et al. 2002a), and have consequently resulted in recent advances in stable isotopes analysis into the exploration of potential factors such as age and sex. Using stable isotope ratios in blood and feathers, Bearhop et al. (2006) reported sex-related dietary differences in the South Georgian shag, *Phalacrocorax georgianus,* driven by differences in physiological performances with males tending to dive deeper than females because of their larger size (and hence able to access higher trophic level prey items). These findings are consistent with studies by Lewis et al. (2015) on the contrasting foraging behaviour of male and female shags. Isotopic differences are also prevalent between chicks and adults, with chicks commonly exhibiting higher $\delta^{15}N$ values than adults (Hodum & Hobson, 2000, Forero et al. 2002). Such results suggest that adults feed their young with higher trophic level and nutritive value than they feed themselves, although in some cases differences may be linked to unusual fractionation factors determined by differences in growth rates between age classes (Bearhop et al. 2000). This was supported by Harding et al. (2008) who reported that the $\delta^{15}N$ signature of little auk (*Alle alle*) chicks was a result of physiological mechanisms influencing blood $\delta^{15}N$ levels. The stable isotope technique undoubtedly provides a great tool for studying how different life history strategies and physiological processes could determine the magnitude of sex and age differences in seabird species (Forero & Hobson, 2003).

The utility of stable isotope models in determining trophic relationships is predicated upon on there being sufficient quantitative information about the known diet of the consumer, as well as the stable isotope ratio for each of the major prey species comprising of the diet (Herman et al. (2005). Using a catalogue of isotopic data for potential prey in the Weddell Sea, Burns et al. (1997) were able to determine the

dietary specialisation of Weddell seals (*Leptonychotes weddellii*) in McMurdo Sound on *Pleuragramma antarcticum.* However, similar $\delta^{15}N$ values between potential prey items led the authors to conclude that without the scat samples and earlier isotopic findings of Rau et al. (1992), the dietary specialisation could not have been determined by the isotope data alone, therefore by combining techniques it was possible to more completely characterise the diet of Weddell seals than would otherwise have been possible. These views were consistent with that of Hobson & Welch (1992) and more latterly Sydeman et al. (1997) who found that in the absence of complete isotope data for the seabird community, additional dietary information provided by conventional methods improved the evaluation of trophic relationships and temporal changes in the diets of seabirds, particularly in ecosystems characterised by extensive changes in physical oceanographic processes.

Moreno et al. (2011) suggested that some of these difficulties with the stable isotope approach results from dietary and trophic level overlap of prey. Due to their position at the top of most food webs, higher vertebrates generally share a small number of common food resources of similar trophic level (Forero et al. 2004). Hodum & Hobson (2000) for example, reported considerable inter-specific overlap in $\delta^{15}N$ values and thus relative trophic positions amongst four petrel species in the Antarctic; results consistent with those of Rau et al. (1992) who found extensive overlap in $\delta^{15}N$ values for seabirds, seals and marine mammals in the Weddell Sea. Using isotopic data for potential prey in the Antarctic and findings from existing studies, Hodrum & Hobson were able to determine that Antarctic petrels exploit a small number of trophic levels and a small subset of prey species. Such results are consistent with a food web comprising of a few trophic steps and most likely a consequence of a super abundance of prey locally but of relatively low diversity (Cherel et al. 2000).

Additional factors that have been highlighted as potentially affecting the utility of stable isotopes in establishing trophic relationships, is the nitrogen balance of the organism (Fuller et al. 2005). Because starving animals, or those in nutritional stress literally “live off their own meat” (Waterlow et al. 1978), the processes by which their tissues become enriched in ^{15}N are the same as those causing trophic level nitrogen

fractionation. Voigt et al. (2003) observed unpredictable enrichment in ^{15}N in nectar-feeding bats which they attributed to the excreted 'lighter' nitrogen not being replaced by dietary protein, resulting in the animal becomes progressively more ^{15}N enriched within increased stress. Similar findings were also found in bears, birds, and pregnant women (Fuller et al. 2005), highlighting that it is not possible to assume that there is a constant ~3% diet-fractionation factor for N, and that the protein content of the diet and nitrogen balance of an organism must be considered so that correct inferences about unknown dietary habits can be made (Gannes et al. 1997; Fuller et al. 2005).

1.3.3 Temporal variation and tissue turnover

A fundamental advantage to using the stable isotope approach over more conventional means of dietary analysis is the *time integrated* nature of the technique (Hobson & Clark, 1992a, Sponheimer et al. 2003). Many animals show a degree of temporal variation in their diet at some point in their life cycle, either as seasonal variation or as a long-term effect over many years (Dalerum, 2005). Anadromous Pacific salmon for example, begin their life in freshwater streams, then undergo smoltification and migrate downstream to estuaries and then the open ocean, subsequently consuming foods with very distinct isotopic signatures (Phillips & Eldridge, 2006). Conventional methods of diet analyses alone can only provide a snapshot of the most recent meal at the time of sampling and subsequently do not have the power to understand or distinguish such patterns of change unless repeated over a period of time (Thompson et al.1995; Sydeman et al. 1997). Stable isotopes can, however, reconstruct such dietary patterns, thus offer the opportunity to answer questions about how an animal forages or distributes itself either at a particular period in its life history or over a much longer period (Gannes et al. 1998).

The isotopic signature of a consumer's tissue generally reflects its diet throughout the period of tissue synthesis (Minagawa & Wada, 1984; Lajtha & Michner, 1994; Ruiz-Cooley, 2004). Since many animal tissues are in a continual state of degradation and renewal, the dietary information that a specific tissue can provide is dependent on its metabolic turnover rate (Phillips & Eldridge, 2006). Several studies have attempted to determine the rates of tissue turnover for a variety of animals,

including birds, mammals and marine organisms (Table 1.3). In some of the earliest work, Hobson & Clark (1992) determined the turnover rates of blood, muscle, bone collagen and liver in quail and reported turnover rates between 2.6 and 173 days, whilst similar studies by Tieszen et al. (1983) showed comparable turnover rates, although slight inter-tissue variations were thought to be a result of growth. Aycliffe et al. (2004) summarized published literature on metabolic turnover rates in a variety of taxa and grouped tissues into three broad categories: metabolically active tissues with turnover rates of a matter of hours to a few days (e.g., blood, liver, faeces, plasma); tissues with turnovers of 10s to 100s days (e.g., feathers, muscle) and those that can take many years (e.g., bone collagen).

Table 1.3. Tissue turnover rates for a range of animal species

Animal	Tissue	Turnover rates (days)	Reference
Japanese quail, *Coturni japonica*	Blood Liver Muscle Collagen (bone)	11.2 (all ^{13}C) 2.5 12.4 173.3	Hobson and Clark, 1992
Great skua, *Catharacta skua*	Blood	15.1 (^{13}C) 12.0 (^{15}N)	Bearhop *et al,* 2002
Garden warbler , *Sylvia borin*	Blood	5.4 (^{13}C)	Hobson and Bairlein, 2003
Sperm whale, *Physter macrocephalus*	Skin	75 (^{13}C)	Ruiz-Cooley, 2004
Mouse, *Mus musculus*	Blood	18.6 (^{13}C) 19.6 (^{15}N)	MacAvory *et al,* 2006

While the factors that drive tissue turnover rates are poorly understood, the literature generally supports the assumption that tissue turnover rates are related to an organism's metabolic rate (Tieszen et al.1983; Hobson & Clark, 1992; MacAvoy et al. 2006). Furthermore, studies continue to show that significant difference exist between organisms and tissues in how quickly they incorporate and retain isotopic signatures Gannes et al. (1998). For example, MacAvoy et al. (2006) suggested that turnover rates vary among species due to the effect of body size over metabolic rate, although other factors including age, growth and nutritional stress of the consumer have also been suggested (Overman & Parrish, 2001). This uncertainty therefore highlights the fact that more experimental work on turnover rates of stable isotopes in different tissues is needed for all taxa in a variety of controlled and wild settings (Gannes et al. 1998). However, it is important to highlight the observations of

Hobson and Clark (1992a, 1993) and more latterly Bird et al. (2003) that the metabolic rates of captive animals can vary substantially from those in the field, and hence relationships and rates derived in captive studies may not be accurate models to apply to the diets of free ranging animals.

1.3.4 Diet – tissue fractionation

A fundamental limitation of the stable isotope approach is that studies continue to show that significant differences exist between organisms and tissues in their isotopic values (Gannes et al.1998). The isotopic ratios of nitrogen and carbon within food fractionate change when incorporated into a consumer's tissue as a result of a variety of biochemical processes (DeNiro & Epstein, 1978; Burns et al. 1999). However, the direction and magnitude of this change and subsequently the dietary or trophic information obtained is ultimately dependent on the tissue or product analysed as well as the body condition or nutritional stress of the consumer, although other factors including species, age and sex can also influence ratios (Gannes et al. 1998). Furthermore, interpreting patterns of stable isotope ratios in consumers to determine dietary sources requires an understanding of the isotopic signature of potential food items, how they fractionate once assimilated and how isotopes become incorporated into different tissues (Petersen & Fry, 1987).

Because isotopes are routed differentially to specific tissues and body components (Owens, 1987; Gannes et al. 1997) through the process of 'isotopic routing,' tissues generally do not reflect the isotopic composition of the bulk diet, but rather the isotopic composition of the component of the diet from which the tissue was synthesized. The composition of body protein in omnivores, for example, often reflects the isotopic composition of dietary protein (Ambrose & Norr, 1993) thus the fractionation associated with these components must be understood for accurate interpretation of diet (Petersen & Fry, 1987; Gannes et al.1997). However, our understanding of these processes is still not complete, and while the utility of the stable isotope technique is valid, it is clear that there is a need, as indicated by Moreno et al. (2011) for more laboratory investigations. In particular, there is a growing need for more experimental data to interpret the growing body of information on the isotopic composition of animal tissues, and to determine how this composition

is related to the animal's dietary constituents (Fuller et al. 2005). Without such data, the interpretation of relationships between the isotopic composition of diets and consumers gathered in the field will remain tenuous. For example, Fuller et al. (2005) observed that an important benefit of such experiments is that data gathered will shed light on how animals allocate nutrients and nutrient components to different tissues, which is a long standing question in ecological physiology.

1.4 Heavy metal concentrations in breeding seabirds.

Since seabirds spend most of their time in the marine environment where they are exposed to a wide range of chemicals and occupy relatively high trophic levels, they are susceptible to bioaccumulation of pollutants and as a result are regularly used to monitor heavy metal concentrations in marine food webs (Schreiber & Burger, 2002; Dauwe et al. 2004; Burger et al. 2007, Kunito et al. 2008, Michelutti et al. 2010; Jepson et al. 2016). The major groups of pollutants of concern are pesticides, metals, chlorinated hydrocarbons, and petroleum products, although more recently attention has focused towards a much wider range of industrial and agricultural compounds that may interact with the endocrine system or have hormone-like effects (Burger & Gochfeld, 2004). While the method of exposure will vary between avian and mammalian species, for seabirds ingestion of food and water are the main routes of exposure (Burger 1993, Jakimska et al. 2011).

Since pollutants tend to bio-accumulate in the tissues of seabirds, many toxins have been found in seabirds, with some implicated in the suppression of the immune system (Schreiber & Burger, 2001) lowered reproductive success (Becker et al. 1993, Kunito et al. 2008), increased susceptibility to disease or other stresses, and changes in behavioural patterns (Scheuhammer, 1987). Concentrations of heavy metals in seabirds, in particular cadmium, lead and mercury, have been reported extensively in the literature (Braune, 1987; Monterio et al. 1998; Stewart et al. 1999; Becker et al. 2002, Kaur Kler et al. 2014), although detecting their deleterious effects has proven difficult. Many seabirds exhibit relatively high concentrations of cadmium and mercury, far in excess of those known to be harmful to terrestrial birds, with apparently little or no effect (Monterio & Furness, 1997; Thompson & Hamer, 2000). For example, in a study using feathers as a monitoring medium, Thompson et al.

(1991) found no relationship between mercury concentrations and a wide range of reproductive parameters in great skuas (*Catharacta skua*), leading Furness (1996) to suggest that the threshold level above which adverse effects occur in pelagic seabirds is most probably higher than for other birds. Table 1.4 provides a summary of the sub-lethal effects associated with elevated concentrations of metals in birds.

Table 1.4. Sub-lethal effects associated with elevated concentrations of metals on birds.

Metal	**Toxicological effects**
As	Suppression of immune system; paralysis; embryo deformities and abnormalities.
Cd	Growth retardation and weight loss; anaemia; suppression of immune system; reduced egg production.
Co	Growth retardation and weight loss; suppression of immune system.
Cu	Growth retardation and weight loss; anaemia; lesions; gizzard damage; reduced egg production; embryo deformities and abnormalities.
Fe	Growth retardation and weight loss.
Pb	Growth retardation and weight loss, anaemia; suppression of immune system; decreased bone density; damage to nervous system; paralysis; abnormal skeletal development; reduced egg production and hatchability.
Mn	Growth retardation; damage to nervous system; decreased fertility; embryo deformities and abnormalities.
Ni	Growth retardation and weight loss; decreased bone density.
Se	Growth retardation and weight loss; anaemia; lesions; reduced egg production and hatchability; embryo deformities and abnormalities.
V	Growth retardation and weight loss; reduced egg production.
Zn	Growth retardation and weight loss; reduced egg production and hatchability.

The exposure to, and specific nature of the pollutant will determine whether it causes an effect since different families of seabirds and different species within these families, have different life history strategies, behaviour and physiology, ecology, dietary habits and habitat use which will mean that their respective responses or vulnerability to a pollutant will vary (Burger & Gochfeld, 2004, Kaur Kler et al. 2014).

For example, Kunito et al. (2008) found that levels of arsenic in the livers of black-footed albatross (*Phoebastria nigrripes*) were more than double that of the black-tailed gull (*Larus crassirostris*) emphasising the significant interspecific differences in levels of heavy metals which can be explained predominantly by differences in dietary habits with birds at higher trophic levels accumulating metals more intensively (Kaur Kler et al. 2014). Furthermore, susceptibility of a species may also vary with age, reproductive stage and gender of the individual although this approach may not be consistent across all pollutants (Lucia et al. 2010, Kaur & Dhanju, 2013). For example, Barbieri et al. (2006) reported significantly higher concentrations of cadmium and lead levels in the kidney tissue of adult Great shearwaters (*Puffinus gravis*) compared to juveniles as a result of bioaccumulation with age, a pattern also reported by Lucia et al. (2010) in the feathers of aquatic birds of the south west of France. That said, contaminate burden in feathers is often greater in juveniles as opposed to adults as a result of the moulting process in adults which enables the removal and excretion of heavy metals (Kaur & Dhanju, 2013).

From the many sampling techniques employed to use seabirds as monitors of pollution in the marine environment, much focus has been given to the development of non-invasive methods that do not involve the killing or biopsy of animals (Furness, 1993). Birds have several methods of ridding the body of contaminants, including normal excretion via faeces, deposition in the uropygial gland or salt gland (Kaur Kler et al. 2014) and sequestering them in eggs or feathers (Burger, 1994; Burger & Gochfeld, 2000a), with some of these materials being widely used as indicators of heavy metals in seabirds (Monterio et al. 1998). However, each of these methods has its own limitations and one method may be more suited than another to measure the particular parameter being monitored.

These earlier studies have tended to focus on the heavy metals, whereas the present study broadens out also to investigate the stable isotopic characteristics In the expectation that they may constrain some of the controls on heavy metal enrichment or depletion.

1.5 Guano (faeces) as a medium for monitoring change in the marine environment

Historically, most stable isotope studies of vertebrates have used bone collagen, muscle, blood and liver to trace dietary and trophic changes (DeNiro & Epstein, 1978; Tieszen, 1983; Dalerum & Angerbjorn, 2005). However, these tissues generally result in the killing or biopsy of the animals being studied therefore new methods for non-destructive sampling are always being developed (Furness, 1993). Indeed, more recent investigations have shown that a great deal of dietary information can be obtained from the isotopic analysis of non-invasive tissues such as feathers, hair, eggs, claws, skin and faeces, thereby reducing the need to sacrifice animals (Bearhop et al. 2003; Sponheimer et al. 2003; Codron et al. 2012; Tsutaya et al. 2016).

A study by Thompson & Furness (1995) found that breast feathers showed comparable results to bone collagen and could be used as a more non-invasive method, while blood plasma has increasingly been used to infer short-term (days) diet in a range of animals (Hodum & Hobson, 2000; Forero et al. 2002; Martins et al. 2012). Furthermore, the increasing trend towards understanding dietary patterns of animals over time has meant that multiple tissue sampling has been increasingly adopted in ecological studies (Sydeman et al. 1997; Bearhop et al. 2000; Podlesak et al. 2005). For example, there is a growing body of literature in which stable isotope analysis of metabolically inert tissues (e.g., feathers, skin, hair, claw, baleen) have been combined with analyses of blood to answer specific questions about migration patterns, seasonal variation in diet and foraging specialisation in a wide range of animals (Bearhop et al. 2000; Forero et al. 2002a, Forero & Hobson, 2003).

While non-invasive tissues such as feathers, claws and eggs have proven general utility in dietary studies, they all require repeated disturbance to animals, particularly if a time series of data is required (Bearhop et al. 2003). The use of excreta as an indicator is a potentially very useful non-invasive method of obtaining information on dietary patterns of birds. While many studies with birds have focused on dietary patterns (Quillfeldt et al. 2005), trophic relationships (Sydeman et al. 1997; Bocher et al. 2000) and pollutant burdens (Metcheva et al. 2011; Celis et al. 2012 & 2014)

utilising stable isotopes, investigations into the use of excreta to address some of these issues have been limited, and further research is required in this area.

1.5.1 Excreta

A fundamental advantage of excreta over other tissues is that large number of samples can be collected from multiple species over extended periods of time without causing unnecessary stress or disturbance to the animal. Excreta generally reflect an animal's consumption over a relatively short period of time (e.g., within days) given the high rate of digestion typical of birds (Hilton et al. 2000). This therefore enables researchers to investigate short-term dietary fluctuations (Codron et al. 2005) which can be repeated over a multitude of timescales without time consuming and expensive observation studies (Codron et al. 2005, Kaur & Dhanju, 2013).

Many of the studies utilising excreta have focused on reconstruction of diet through the identification of hard parts from prey such as bones, scales, eggs or otoliths of fish, all which may survive digestion and are often excreted (Barrett et al. 2007). This method has been applied to many different mammalian piscivores, most notably pinnipeds and otters (Tollit et al. 1996; Andersen et al. 2004; Wilson et al. 2016), but very limited studies have been carried out on seabirds (e.g., omnivorous gulls and skuas) because very few hard parts are present in seabird excreta (or guano). Such methods however, are often biased due to their differential rates of digestion (Phillips et al. 2005) or due to the fact that birds often regurgitate hard parts via pellets (Barrett et al. 2007) and are therefore are unlikely to reveal all prey taken by the predator. Moreover, some parts survive better than others and some prey may be completely overlooked or greatly underestimated. As a result, other analytical methods (e.g., fatty acid analysis, stable isotope analysis, DNA extraction) using excreta as the tissue of choice have been investigated to attempt to overcome some of these limitations (Owen et al. 2013).

Over recent years, many of the studies that have used stable isotope analysis of excreta to make inferences about an animal's diet generally focus on mammalian vertebrates, including impala (Sponheimer et al. 2003), gorillas (Gustine et al. 2012)

and deer (Najera-Hillman & Mandujano, 2013) with more limited studies undertaken on birds (Mizutani & Wada, 1988 a & b; Podlesak et al. 2005, Bird et al. 2008). These experimental and field studies have demonstrated that faecal stable isotope compositions are generally consistent with diet isotope compositions of the animal (Codron et al. 2011). Yet since faeces are comprised of undigested food remains and various endogenous losses (e.g., gut microbes, waste metabolic products, digestive secretions and tissues), variations in their stable isotope composition may be derived from non-dietary effects (Codron et al. 2012). Indeed, faeces-diet discriminations reported in the literature vary substantially between independent studies and within them (Sponheimer et al. 2003, Codron et al. 2011) supporting the widely held view that variations in animal-diet discriminations in any type of material are the biggest constraint for accurate and reliable diet reconstructions by stable isotope analysis. This hypothesis was supported by Codron et al. (2012) who found that while ^{13}C in excreta was a reliable proxy for determining wildlife diets, further work on factors influencing ^{15}N abundance is required. In contrast, both Blumenthal et al. (2012) and Bird et al. (2008) found a strong correlation between the diet and faecal stable isotopic compositions of both ^{13}C and ^{15}N in wild mountain gorillas and zebra finches respectively leading them to conclude that stable isotope analysis of faecal material is potentially a powerful tool and one that requires further investigation for dietary studies.

1.5.2 Uric acid

To avoid some of the potential constraints and conflicting studies with using faecal material as a medium to construct diet, consideration has also been given to the utility of urine or uric acid to fulfill this role (Bird et al. 2008). In human and veterinary medicine urine has a wide range of applications for clinical diagnostic testing, often being used to detect disorders specific to the urinary system (Doxley, 1983). In contrast, its application in wild animals is rarely reported in the literature due to the constraints in its collection, with the exception of snow urine. Snow urine sampling has been reported in wolves, elk and seals (Constable et al. 2006, Hausknecht et al. 2007) in circumstances where urine freezes after being excreted in subzero temperatures, subsequently being preserved for later collection. Such a method however, clearly has limited applications for most wild animals.

In contrast, birds excrete predominantly uric acid as the end product of nitrogen and protein metabolism (Bird et al. 2008), with ammonia and urea making up a much smaller component (approximately < 25% of excreted nitrogen). As uric acid is practically insoluble in water, it is excreted in a colloidal form with mucus (a white paste like suspension) which as a solid component can easily be collected after the bird has vacated a site and therefore has potential applicability for both field and laboratory studies. Over time uric acid will show a small level of degradation (Mizutani & Wada, 1985 a, b), but this process does not appear to affect the isotopic composition of the remaining uric acid, leading to the conclusion that uric acid preserves the isotopic composition of the food metabolized by the bird and can therefore be used to determine dietary habits.

Several techniques for quantification and isolation of uric acid from urine, and in particular avian excreta, have been proposed (Mizutani & Wada, 1985, Adeola & Rogler, 1994) but no study has investigated the relationship between the stable isotope composition of uric acid and diet until Bird et al. (2008) in conjunction with this PhD, used captive finches to investigate the potential. Using methods adapted from previous literature (Mizutani & Wada, 1985, Adeola & Rogler, 1994), these findings found that when uric acid was extracted from guano, the ^{13}C value of the uric acid reflected the diet of the birds within a matter of days with very little isotopic fractionation, which was comparable to previous isotopic studies using tissues such as blood, liver, collagen and feathers (Hobson & Clark, 1992; Podlesak et al. 2005; Herrera & Reyna, 2007). In contrast, the ^{15}N value of uric acid was partially fractionated (up to 3‰) compared to that of the diet which was surprising considering the ^{15}N value of guano within the same study was strongly correlated to the diet. This led the authors to conclude that this discrimination could possibly be a result of dietary stress experienced by the birds or that ^{15}N was preferentially being excreted as ammonia or possibly urea, a conclusion supported by Sponheimer et al. (2003) through his work on llamas. Such findings led Bird et al. (2008) to conclude that while further work on factors influencing ^{15}N abundance are obviously required, stable isotope analysis of uric acid offers a simple and powerful tool for studying avian ecology and diet. In particular, it offers potential to provide at much short term dietary information as other tissue types, with the added benefit of much less

invasive sampling techniques and therefore further studies into the utility of this method, particularly using wild samples should be investigated more fully.

1.6 Thesis aims and outline

The above sections have shown that seabirds have a valuable status as sentinels of environmental change due to their life history strategies and the fact that they feed in the upper trophic levels of the marine environment. This combined with their ability to produce collectable guano samples from a wide range of sites over extended time periods which has proven to have potential for answering questions on dietary habits of birds, should make them ideal candidates for monitoring changes in diet, trophic status and pollutant burden of Scottish seabirds.

The aim of this thesis is therefore to develop the utility of seabird guano chemistry for monitoring environmental changes in (i) diet and trophic relationships of selected seabird species, and (ii) anthropogenic pollutant levels with the purpose of testing the hypothesis that guano (and subsequently uric acid) is a valid non-invasive sampling technique. To achieve this overall aim the following specific objectives were identified:

1) Test the utility of seabird guano as a method for investigating seabird diet and trophic relationships through isotopic analysis.
2) Further develop a methodology for extracting uric acid from avian guano for isotopic analysis.
3) Investigate the stable isotope composition of both guano and uric acid in some seabird species on a temporal and spatial scale.
4) Investigate heavy metal concentrations in the guano of breeding seabirds across Scotland to determine the utility of guano as a potential indicator.

Each of these objectives will be addressed separately although there will be some overlap between these themes.

Chapter 2. Sampling of seabird populations: Study sites and sampling methods.

2.1 Study sites

Samples were collected from ten seabird colonies around the Scottish coast (Figure 2.1) during the breeding season (April to August) in four years between 2006 and 2009, with varying frequency. The following section provide more detailed information on the sampling locations, time periods and species studied.

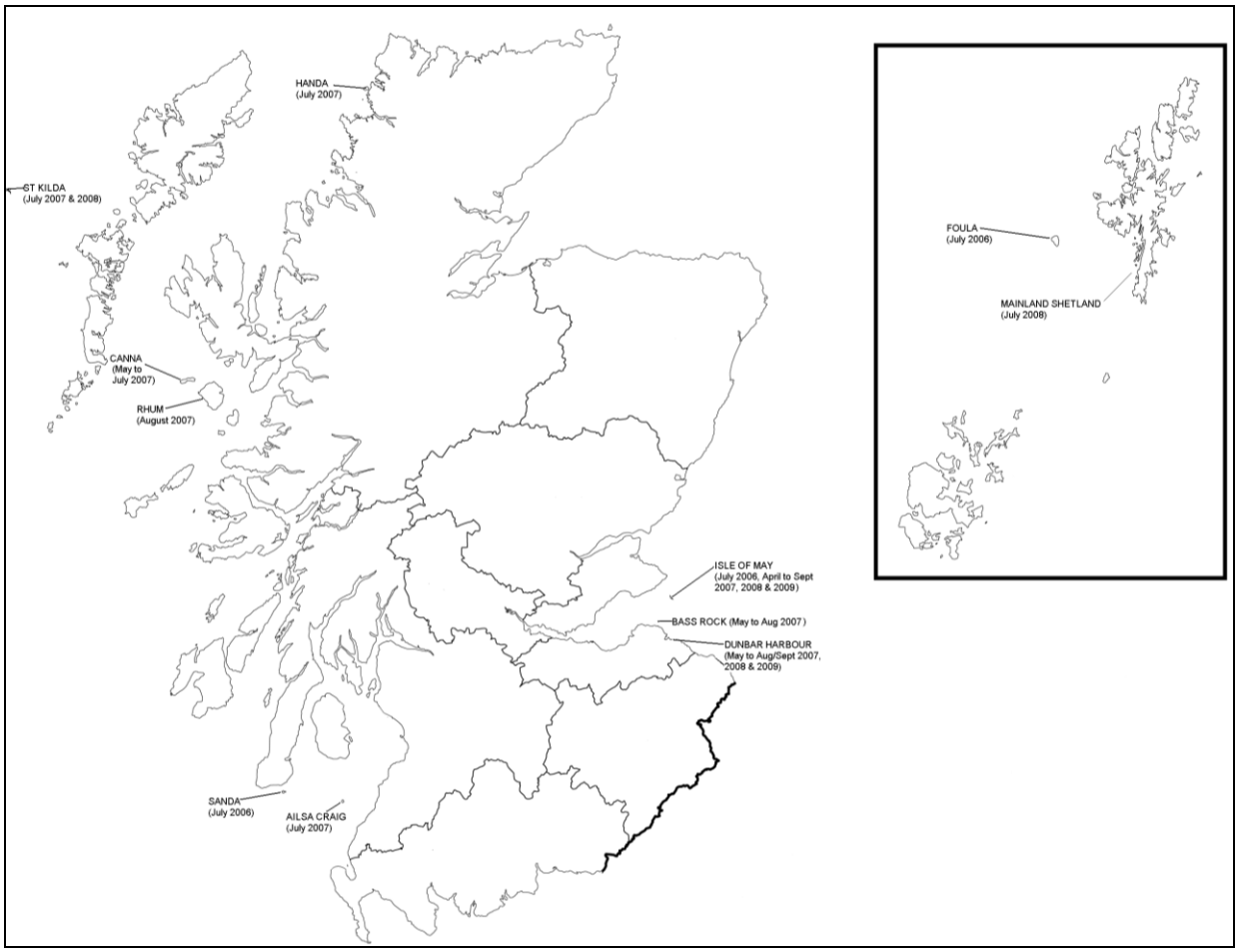


Figure 2.1. Sampling locations around the Scottish coast

2.1.1 Isle of May

Located in the outer Firth of Forth, southeast Scotland, the Isle of May lies approximately eight kilometres from the Scottish mainland (56.18^{O}N; 2.55^{O}W) and is owned and managed by Scottish Natural Heritage (SNH) as a national nature reserve. The island is one of the most important seabird breeding colonies on the

British North Sea coast, supporting populations of Atlantic puffins *Fratercula arctica,* northern fulmars *Fulmarus glacialis*, European shags *Phalacrocorax aristotelis*, common guillemots *Uria aalge*, razorbills *Alca torda,* black-legged kittiwakes *Rissa tridactyla*, herring gulls *Larus argentatus,* lesser black-backed gulls *Larus fuscus*, common terns *Sterna hirundo* and arctic terns *Sterna paradisaea,* with most of these populations being of international importance (Mitchell, 2004). Due to its importance for breeding seabirds, the Isle of May forms part of the Firth of Forth Islands Special Protection Area (SPA) along with breeding colonies at Inchmickey, Fidra, Lamb, Craigleith and Bass Rock.[1]

Seabird populations have been studied continuously on the Isle of May since 1972.[2] The Isle of May is one of four key UK seabird monitoring sites supported by the Joint Nature Conservation Committee's (JNCC) Seabird Monitoring Programme, and the Centre for Ecology and Hydrology (CEH) has an ongoing contract to collect basic data on seabird performance (e.g., adult survival, breeding success and diet) with the aim of assessing the status of their breeding populations and to monitor the state of the marine environment (Wanless et al. 2007). The Isle of May long-term study (IMLOTS) is currently one of the most data-rich and comprehensive studies of its type in Europe.

Black-legged kittiwakes *Rissa tridactyla* are one of the five key species monitored as part of the Isle of May long-term study, and for the purpose of this thesis are the species of focus for data collected on the island. Kittiwake populations in the North Sea have fluctuated widely since 1990 reaching their lowest level for 17 years in 2008 (Newell et al. 2013b), probably a result of successive years of poor breeding success due to low availability of their preferred food source coupled with low survival and return rates to colonies (Frederiksen et al, 2004 & 2007). Over the last two decades, the number of breeding pairs of kittiwakes on the Isle of May has fallen from 8129 pairs in 1990 to 2464 pairs in 2014 (Figure 2.2 a). Breeding success has fluctuated during this time with no linear trend, and in 2014 kittiwakes had one of

[1] http://jncc.defra.gov.uk/page-1970

[2] www.ceh.ac.uk/our-science/projects/isle-may-long-term-study

their most productive years since recording began with an average of 1.17 chicks produced per breeding pair (Scottish Natural Heritage, 2015) (Figure 2.2 b).

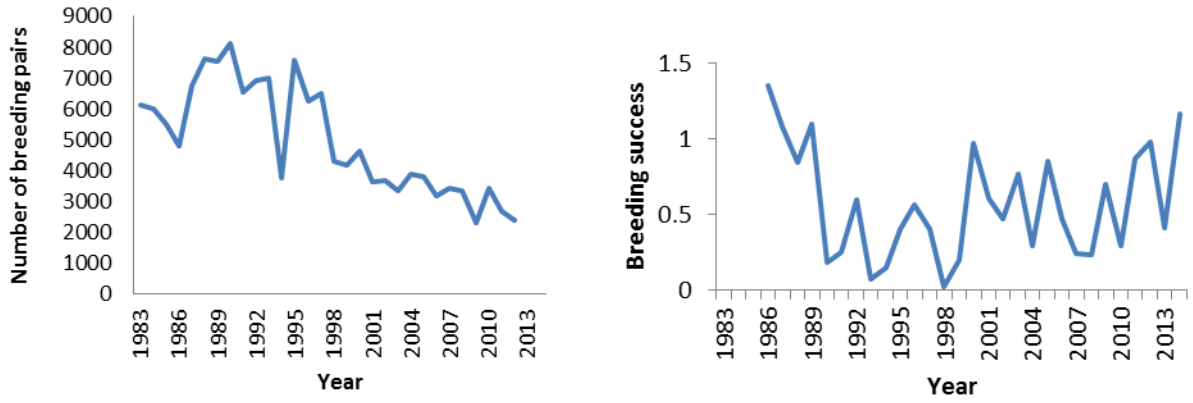


Figure 2.2 (a) Number of breeding pairs and **(b)** mean breeding success of kittiwakes on the Isle of May (1983-2014 and 1986-2014 respectively).

Sampling locations

Kittiwakes nest all around the Isle of May both on the high cliffs of the western side and on the lower rocky eastern side. Many of the nest sites on the island can be accessed with an extended noose pole, and to date much information has been collected on kittiwake diet, breeding success, feeding and foraging behaviour using this method (Daunt et al. 2002, Wanless et al. 2007). However, due to the need to access nest sites by hand to collect fresh guano samples, sampling was restricted to more accessible nest sites at Colm's Hole (Figures 2.3-2.5) and Ardcarron gulley (Figure 2.6). Since kittiwakes in these locations have been intensively studied over the years, we were able to get close to the breeding birds and young chicks without causing flight from the nest site.

Located to the north east of the island, Colm's Hole (56.11^{O}N; 2.33^{O}W) is a shallow rocky inlet which opens up into a wide grassy basin. Kittiwakes nest on the wall of the inlet ('left gulley') and on rocky faces to the south ('back gulley) and east ('right gulley') of the basin. All three sampling sites were easily accessible by foot and the individual nest sites selected on the basis that they were (a) sheltered enough to avoid guano deposits from other birds, (b) positioned to reduce potential for

disturbance to incubating birds (on the nest and surrounding nests), and (c) had no other seabird species nesting nearby.

Located to the eastern tip of the island, Ardcarron Gulley (56.10^{O}N; 2.32^{O}W) is found at the head of a rocky inlet, which widens out into a shallow grassy plateau. Kittiwakes nest in small numbers on the rocky walls of the inlet, approximately two metres above the inlet floor. Other seabird species such as shags and razorbills nest to the seaward side of the inlet, but not in close proximity to the kittiwakes. Like Colm's Hole, all nest sites are easily accessible with minimal disturbance to the nesting birds. Five nest sites were sampled during the course of the breeding season (Table 2.1).

Figure 2.3. Left Gully, Colm's Hole, Isle of May.

Figure 2.4. Back Gully, Colm's Hole, Isle of May.

Figure 2.5. Right Gully, Colm's Hole, Isle of May.

Sampling time period

Sampling was undertaken at the Isle of May during the 2007 breeding season between the months of April and July. To assess changes in the isotopic signature of guano over the course of the breeding season, four time periods were defined, each spanning approximately three days: 20-22 April (early breeding season), 16-17 May (egg incubation), 18-19 June (feeding chicks) and 23-24 July 2007 (fledging/late breeding season). Limited sampling was also undertaken in the 2008 and 2009 breeding seasons to look for any inter-annual changes in the isotopic composition of guano, and subsequently the diet of the kittiwakes. Table 2.1 provides information on the number of nest sites and samples collected for each location.

2.1.2 Dunbar Harbour

Located within the Firth of Forth on mainland Scotland (56^{O}'N, 2O31'W), the ruins of Dunbar Castle are home to the largest man-made blacked-legged kittiwake colony in Scotland. Due to the accessibility of the nest sites, this colony has a well-documented history starting in 1934 when three nests were recorded (Coulson, 1963). Since then the colony has shown a steady increase in breeding numbers probably due to the availability of a large number of nesting sites (Coleman et al. 2011).

East Lothian Council's Countryside Ranger Service monitors the kittiwakes and their changing populations by undertaking counts of the occupied nest sites and productivity on an annual basis (East Lothian Council Ranger Service, *pers comms*). Between 1990 and 2007 the number of nest sites at Dunbar Harbour increased from 577 occupied nests in 1990 to 1,155 nests in 2007 (Figure 2.6 a) while breeding success has varied significantly during this time with no linear trend observed.

The main kittiwake colony is located on the old castle wall and on the rock face at the entrance to the harbour, although satellite nest sites can be found on the colony edge. Approximately 90% of all the nests are accessible using ladders, but for the purpose of this project nest sites accessible on foot were selected on the south and east facing castle walls.

Table 2.1. Table showing (a) number of samples, (b) number of nest sites and (c) the unique identifiers for kittiwakes breeding on the Isle of May and at Dunbar Harbour between 2007 and 2009.

Location	Number of nest sites (by year)	Number of samples (each year)	Unique identifier
Isle of May			
Colm's Hole:			
Left Gulley			
2007	9	50	KW-CHL-101
2008	9	14	KW-CHL-201
2009	6[1]	11	KW-CHL-301
Right Gulley			
2007	4	9	KW-CHR-101
2008	2[2]	4	KW-CHR-201
2009	5[3]	8	KW-CHR-301
Back Gulley			
2007	5	33	KW-CHB-101
2008	4[4]	7	KW-CHB-201
2009	6[5]	10	KW-CHB-301
Ardcarron			
2007	8	32	KW-ARD-101
2008	4[6]	9	KW-ARD-201
2009	7[7]	16	KW-ARD-301
Dunbar Harbour			
2007	9[8]	65	KW-DUN-101
2008	7[9]	31	KW-DUN-201
2009	7	30	KW-DUN-301

[1] Three nest sites (1, 7 and top nest) were occupied in 2009. No new sites selected for guano collection as no suitable ones were available.

[2] Nest 3 and far nest not occupied in 2008. No new sites selected for guano collection as no suitable ones were available.

[3] Nest 3 and far nest occupied again in 2009. An additional nest (nest 4) added for additional guano collection.

[4] Nest 3 not occupied in 2008. No new sites selected for guano collection.

[5] Nest 1 not occupied in 2009. An additional two nest sites (nests 6 and 7) added for additional guano collection.

[6] Nest sites 1 to 4 not occupied in 2008. No additional nest sites selected for guano collection.

[7] Nest sites 1 and 2 occupied again in 2009. One additional nest site selected.

[8] Although 9 nest sites were selected, additional samples were collected from the east face in August by placed a plastic sheet on the ground beneath the colony and collecting the deposited guano.

[9] Nests 4 and 6 not occupied in 2008 and 2009. No new nest sites selected.

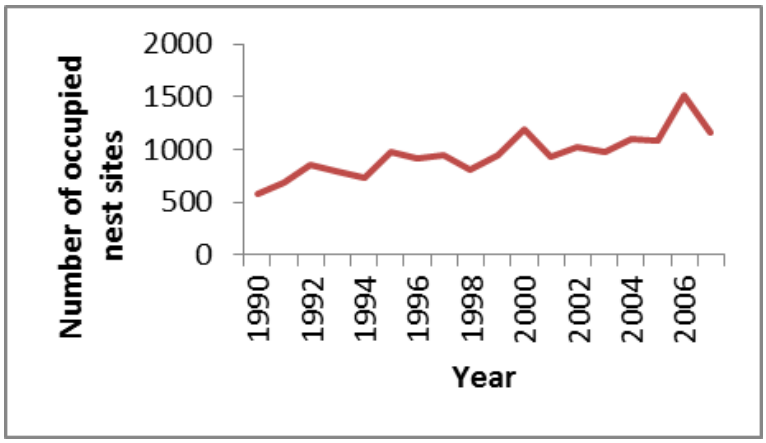


Figure 2.6 (a) Number of occupied nest sites of kittiwakes at Dunbar Harbour (1990-2007).

Sampling time period

Sampling was undertaken at Dunbar Harbour during the 2007 breeding season between the months of May and August. To assess changes in the isotopic signature of guano over the course of the breeding season, four time periods were defined, each spanning approximately 3-5 days: 17-22 May (egg incubation), 25-27 June (feeding chicks), 22-26 July and 12-15 August 2007 (fledging/late breeding season). Limited sampling was also undertaken in the 2008 and 2009 breeding seasons to look for any inter-annual changes in the isotopic composition of guano, and subsequently the diet of the kittiwakes. Table 2.1 provides information on the number of nest sites and sample, and unique identifier collected for each location.

2.1.3 Other colonies

As different seabirds feed in, and therefore 'sample' different habitats, it is likely that there are significant species-specific differences in the isotopic composition of guano which reflects these different food sources. To assess the isotopic signature of different seabird species from a diverse range of geographical locations, 'one off' sampling was undertaken at breeding colonies around the Scottish coast (Figure 2.1) in 2006, 2007 and 2008 providing a good spatial spread of data from regions of differing oceanography and climatology. Table 2.2 provides information on the sampling location, species and time frames.

Bass Rock

Located in the outer Firth of Forth, Bass Rock (56^{O}05'N 02^{O}36'W) is one of the most important breeding colonies in the world for Northern gannets *(Morus bassanus)*, with over 150,000 pairs attending the island each year making it the largest gannet colony in the world (Murray et al. 2014). The Centre for Ecology and Hydrology has been carrying out research on Bass Rock's gannets over the last thirty years, monitoring many aspects of their biology (Hamer et al. 2000 & 2001; Camphuysen, 2011) to assess the status of their breeding populations. Gannets have not been subject to the recent decline witnessed by other species, with populations on the Bass Rock showing an overall increase of 24% since the last count in 2009 (Wanless et al. 2015). This is probably due to their ability to forage far for food and being able to sample a wide range of food sources (Camphysen, 2011). The sea cliffs of Bass Rock are also home to other seabird species such as kittiwakes, guillemots and puffins and forms part of the Firth of Forth Islands Special Protection Area (SPA)[3]. Samples were collected from Northern gannets (*Morus bassanus)* and herring gulls (*Larus argentatus).*

Rhum

Located 15 miles off the west coast of Scotland, Rhum (57^{O}00'N 06^{O}17'W) is the largest of the Scottish Small Isles, with an area of approximately 105 km^{2}. Owned and managed by Scottish Natural Heritage, Rhum supports more than 130,000 seabirds during the breeding season, including the world's largest colony of Manx shearwaters - at least 23% of the world's breeding population (SNH, 2009). The islands sea cliffs are also home to other internationally important seabird species, including kittiwakes, guillemots and razorbills, and is designated as a Special Protection Area for its seabird assemblages (SNH, 2008). Seabird populations on the island have been largely stable, although it has been suggested that the shearwater colony is in slow decline, possibly due to increasing levels of rat predation causing reduced breeding success (Furness, 1997; Lambert et al. 2015). Samples were collected from black-legged kittiwakes (*Rissa tridactyla)* and Manx shearwaters (*Puffinus puffinus)*.

[3] http://jncc.defra.gov.uk/page-1970

St Kilda

The St Kilda archipelago (57^{O}53'N 08^{O}30'W) consists of four islands and a number of smaller rock stacks and lies 66km west of North Uist in the Western Isles. Owned and managed by the National Trust for Scotland (NTS), St Kilda is the largest seabird colony in the northeast Atlantic with over one million birds attending the archipelago each year (National Trust for Scotland, 2014). The islands are recognised by UNESCO as a dual World Heritage Site, and are designated as a Special Site of Scientific Interest (SSSI), Special Protection Area (SPA) and a Special Area of Conservation (SAC) for the assemblages of seabirds that breed on the islands (Mitchell, 2004). St Kilda is home to internationally important populations of Northern gannets, Atlantic puffins, great skuas and Leach's petrel, with the latter accounting for 89% of the world biogeographic population (National Trust for Scotland, 2014). Recent counts indicate a significant decline in fulmar (19%), common guillemot (14%) and kittiwake populations on the islands since 2011, while number of gannets remain relatively stable, probably a result of strong density dependent effects (Wanless et al. 2015). This thesis focuses on the analysis of guano samples from great skuas, (*Catharacta skua)*.

Ailsa Craig

Located in the entrance to the Firth of Clyde (55^{O}15'N 05^{O}08'W) ten miles west of the Ayrshire coast, Ailsa Craig's sea cliffs are home to over 73,000 breeding seabirds, including 36,000 pairs of northern gannet, *Morus bassanus* making it the third largest gannetry in the UK. Unlike other colonies around the UK, gannet numbers at Ailsa Craig have decreased over the last decade, possibly as a result of a reduction in adult survival (Wanless et al. 2015). Other species of seabirds also nest on the island, including guillemots, razorbills, kittiwakes and increasingly puffins. Due to its importance for seabirds, Ailsa Craig is designated as a Site of Special Scientific Interest (SSSI) and a Special Protection Area (SPA) and is managed as a bird reserve by the Royal Society for the Protection of Birds (RSPB). As part of this thesis, guano samples were taken from the Northern gannet (*Morus bassanus)*, lesser black-backed gull (*Larus fuscus)* and herring gulls (*Larus argentatus).*

Sanda

Sanda is located off the southern tip of the Kintyre Peninsula (55^{o}165'N 5^{o}351W) in Argyll and Bute, southwest Scotland. The island is important for migratory and breeding birds, holding good populations of guillemots, razorbills, puffins and Manx shearwater, and is designated as a Site of Special Scientific Interest (SSSI). Studies of the birds of the Sanda group of islands has been taking place over three decades, more recently under the auspices of the Sanda island Bird Observatory and Field Station Trust. Guano samples were taken from common gulls (*Larus canus)* and herring gulls (*Larus argentatus).*

Handa

Located on the north-west coast of Scotland, the sea cliffs and coastal moorland of Handa Island (58^{O}22'N 05^{O}11'W) support nearly 100,000 breeding seabirds, including internationally important numbers of guillemot, razorbill, great and Arctic skua and Atlantic puffin. Since the mid 1980's Arctic skua numbers have declined by over 50%, probably a result of increasing great skua predation and declines in other seabird species such as kittiwakes and auks which Arctic skuas rely on to obtain their food sources (kleptoparatism). In contrast great skuas on Handa have shown good overall productivity with the population increasing slightly each year, although it is probable that the island is close to reaching its carrying capacity (Leckie, 2013). Samples were collected from great skua *(Catharacta skua)* and Arctic skua (*Stercorarius parasiticus).*

Foula

Foula is the most westerly of the Shetland Islands, lying approximately 20 miles west of mainland Shetland (60^{O}07'N 02^{O}03'W). Norse for 'bird island,' Foula has historically supported nationally important numbers of seabird species such as great skuas and European shags, but over the last two decades some populations have shown catastrophic declines. Razorbills and guillemots declined by 95% and 60% respectively between 1976 and 2007, while a photographic survey of the island in 2014 indicates that shags have virtually deserted some areas (Heubeck *et al* 2014). These declines can, in part, be attributed to low sandeel abundance (Malvor *et al* 2006), although other factors such as habitat change, adult predation and competition between species could be contributory factors (Furness 2007, Heubeck

et al. 2014). As part of this thesis, guano samples were taken from black-legged kittiwakes (*Rissa tridactyla),* northern fulmar (*Fulmarus glacialis),* razorbill (*Alca torda),* European shags (*Phalacrocorax aristotelis),* great skua *(Catharacta skua)* and Arctic skua (*Stercorarius parasiticus).*

Burnt Island

Burnt Island is located just off the Fife coast (56^{O}06'N 03^{O}23'W) within the Firth of Forth, southeast Scotland. A variety of gull species occupy and breed in the area.. Guano samples were taken from herring gulls (*Larus argentatus).*

Sampling time period

Sampling was undertaken at each location during the course of the 2006, 2007 and 2008 breeding seasons with the greatest geographical spread collected during a defined two week time period in mid July 2006 when the adults were attending the colony. Table 2.2 presents the locations, species and timescales for sample collection at each of the geographical locations.

2.2 Collection of samples

A total of 911 guano samples were collected from a suite of seabirds from breeding colonies across Scotland. All sampling (from April 2007 onwards) was carried out under a "Bird Science, Research and Education Licence" (number 9864) granted by Scottish Natural Heritage under Section 16 (1) (a) and (c) of the Wildlife and Countryside Act 1981. Sampling undertaken prior to April 2007 was subject to licences granted to the respective research organisations[4].

A unique identifier was attributed to each sample consisting of three parts: Two to four letters identified the species sampled (e.g., KW-kittiwake; GAN-gannet), three letters identified the location (e.g., SAN-Sanda; KIL-St Kilda) and a three digit number identified the sample number in chronological order of sampling based on the calendar year (e.g., KW-RUM-101; KW-DUN-201). Detailed information on the unique identified can be found in Table 2.2.

[4] University of Aberdeen, Centre for Ecology and Hydrology, University of Glasgow

Table 2.2. Location of data collection and species targeted between the 2006 and 2009 breeding seasons.

Year	Location	Species	Unique identifier (no. samples)
2006	Isle of May (July)	Black-legged kittiwakes, *Rissa tridactyla*	KW-IOM-001 25
		Northern shags, *Phalacrocorax aristotelis*	SHG-IOM-001 16
	Foula (July)	Black-legged kittiwakes, *Rissa tridactyla*	KW-FOU-001 21
		Northern fulmar, *Fulmarus glacialis*	FUL-FOU-001 8
		Razorbill, *Alca torda*	RB-FOU-001 8
		European shag, *Phalacrocorax aristotelis*	SHG-FOU-001 25
		Great skua, *Catharacta skua*	GSK-FOU-001 22
		Arctic skua, *Stercorarius parasiticus*	ASK-FOU-001 18
	Sanda (July)	Common gull, *Larus canus*	CGUL-SAN-001 12
		Herring gulls, *Larus argentatus*	HGUL-SAN-001 16
	Burnt Island (July)	Herring gulls, *Larus argentatus*	HGUL-BI-001 15
2007	Bass Rock (May to August)	Northern Gannet *Morus bassanus*	GAN-BR-101 59
		Herring gulls, *Larus argentatus*	HGUL-BR-101 18
	Ailsa Craig (July)	Northern Gannet *Morus bassanus*	GAN-AC-101 22
		Herring gulls, *Larus argentatus*	HGUL-AC-101 15
		Lesser black backed gull	LBB-AC-101 9
	Handa (July)	Great skua, *Catharacta skua*	GSKU-HAN-101 34
		Arctic skua, *Stercorarius parasiticus*	ASKU-HAN-101 12
	Rhum (August)	Manx shearwater, *Puffinus puffinus*	MANX-RUM-101 57
		Black-legged kittiwakes, *Rissa tridactyla*	KW-RUM-101 8
	St Kilda (July)	Great skua, *Catharacta skua*	GSKU-KIL-101 49
2008	St Kilda (July)	Great skua, *Catharacta skua*	GSK-KIL-201 17

2.2.1 Black-legged kittiwakes

Black-legged kittiwakes have well established nest sites to which they return each year in order to breed (Wanless et al, 2005). The nest is a platform made up of mud and vegetation with a cup formed on the top to contain the eggs. The kittiwake pair builds the nest at the beginning of the breeding season often on top of the old nest from the previous year, resulting in nest structures up to 1m in height. These nest structures provide an ideal opportunity to obtain fresh guano samples from a specific species, therefore a sampling protocol was developed for kittiwakes.

Since kittiwakes defecate immediately over the side of the nest, specially designed collection devices were made to collect the deposited guano. These devices consisted of a sheet of acetate, measuring approximately 15cm x 10cm and folded up at the sides, with a wire frame. The wire frame was placed into the nest until the acetate platform fitted snuggly against the nest (Figure 2.8). Two devices were placed on each nest, allowing guano to be collected as it was deposited. This method was successful and allowed individual nest sites to be targeted continuously over the course of the sampling period.

Figure 2.8 Collection device on a kittiwake nest, Dunbar Harbour 2007

The devices were left on the nest for between 12 and 24 hours depending on the time of year. In the early breeding season (April) when the birds are often foraging away from the nest, the sampling period was much longer to allow for guano deposits to be built up. However, during incubation and chick rearing (May and June), a 12 hour sampling period was sufficient due to high attendance at the nest site.

On collection, the devices were placed in a sealable plastic bag, with nest site, location, date and time noted. All samples were frozen within two hours of collection.

2.2.2 Other species

In the case of seabird species which do not have established nest sites (e.g., guillemots, razorbills), or where any material placed close to the nest site for the purpose of collecting guano would be removed as part of the nest building process (e.g., shags, gannets) (CEH, *pers comms*), a sampling protocol was developed whereby guano would be collected from perches of known species. Where possible, five to ten samples per species were collected this way, yielding between 10 and ~200 samples of guano from each location.

All samples were collected and stored in a sealable sample vial or plastic sample bags, noting species location, date and time. Habitual perches on rocks were cleaned between samplings so that only fresh guano was sampled. All samples were frozen at $^{-}18^{o}$C within two hours of collection.

2.3 Transport of samples from field

Movement of frozen samples between the fieldwork locations and the University of St Andrews were carried out using large thermally insulated containers and ice packs. The samples were stored at $^{-}18^{o}$C until required for further analysis.

Chapter 3. Materials and Methods

3.1 Sample preparation

In preparation for analysis, the guano samples were freeze-dried (to -57^{o}C) for a 24 to 48 hour period, passed through a sieve to remove any vegetation and ground in an Agatha (agate) stone mortar to a fine homogenous powder. Samples were subsequently stored in independent glass vials to prevent contamination and exposure to water prior to further analysis. Following this process, the samples were stored in a cool dry location indefinitely.

3.2 Uric acid extraction

The methodology developed for extracting uric acid from seabird guano was adapted from methods described in previous literature (Mizutani and Wada, 1985; Adeola and Rogler, 1994), while the technique for purification of uric acid was adapted from Inoue et al.(1994).

To extract uric acid from bulk seabird guano, 2ml of 0.1M di-potassium hydrogen phosphate (K_2HPO_4) solution was added to a 15ml plastic centrifuge tube containing approximately 50-100 mg of freeze-dried guano and shaken vigorously. To separate the uric acid from the denser faecal material, the solution was shaken and warmed intermittently in a water bath for 10 minutes and then placed in a centrifuge at 3700rpm for 5 minutes. This resulted in the formation of a supernatant containing the extracted uric acid.

Purification of the extracted uric acid was achieved by the process of Solid Phase Extraction (SPE) using a 12-position vacuum manifold and cartridges purchased from Applied Separations (Allentown, PA, USA). This contained a reversed-phase non-polar C18 Octadecyl packing with 500 mg/6mL capacity. Prior to each separation, the SPE cartridge was fully conditioned by drawing through 2ml of methanol followed by 2ml of deionised water. Thereafter, 1.5ml of the supernatant containing the uric acid was drawn through the cartridge followed by 0.5ml of the K_2HPO_4 solution. Uric acid was then precipitated from the eluate by acidifying with 0.3ml of 1M Phosphoric acid (H_3PO_4). At the end of the SPE, the sample was

centrifuged for 5 minutes at 3700rpm. The supernatant was then drawn off and the uric acid precipitate washed and centrifuged in de-ionised water before being freeze-dried for a 24 hour period prior to further analysis.

This method yielded uric acid in excess of 90%, and increased throughput of the SPEs was achieved by the use of a 12-position vacuum manifold. Where required, further purification of the uric acid was achieved by repeating the above procedure.

3.3 Stable isotope analysis

All samples were analysed for C and N elemental abundance and $\delta^{13}C$ and $\delta^{15}N$ values using a Continuous Flow Isotope Ratio Mass Spectrometry (CF-IRMS) at the University of St Andrews Facility for Earth and Environmental Analysis. The instrument was a Costech Elemental Analyser (Valencia, CA, USA) fitted with a zero-blank auto-sampler coupled via a ConFlo III to a Delta Plus XL mass spectrometer (ThermoFinnigan, Waltham, MA, USA) operated in a continuous flow mode. Due to the low C:N ratio of uric acid (1:1) approximately 0.2-0.4mg of ground uric acid was weighed into tin capsules for isotopic analysis. This is in comparison to bulk guano where the generally higher C:N ratios require greater sample weights between 1 and 2mg. Stable isotope results are reported as per mil (‰) deviations from the (Vienna Pee Dee Belemnite) VPDB and AIR reference standard scale for $\delta^{13}C$ and $\delta^{15}N$ values respectively. Precisions on the internal standards used were approximately +/- 0.1‰ and +/- 0.3‰ for $\delta^{13}C$ and $\delta^{15}N$ respectively.

3.4 X-ray fluorescence analysis

3.4.1 Sample preparation

To prepare samples for X-ray fluorescence analysis, 1 and 5g aliquots of finely ground guano was loaded into a 12mm diameter aluminum cup in a stainless steel die and compressed into a flat disc using a hydraulic press machine under a load of 20 tons. Following this process, the pressed discs were stored in a labeled polythene bag prior to further analysis. Where there was insufficient guano samples, samples were combined by species.

3.4.2 Sample analysis

The samples were analysed for heavy metals and some additional metals and metalloids as well as some non-metallic elements using Polarised X-ray Fluorescence Spectrometry (PXRF). Two major batches were run on different occasions and important to note that the PXRF instrumentation was replaced before the second batch. The following description applies to the first batch and different analytical conditions that apply to the second batch are specified separately.

The PXRF instrument was a SPECTO XLAB equipped with a water-cooled 3kW Rh anode tube with 0.5mm Be side window. Detection of fluorescent X-rays was by a Si(Li) detector cooled with liquid nitrogen. Five targets were used, namely Al_2O_3, B_4C, Co, HOPG and Mo. Spectra for each target were counted for between 400 and 600 seconds and the machine was calibrated for the elements Si, P, S, Cl, K, Ca, Ti, Mn, Fe, V, Cr, Co, Ni, Cu, Zn, Ga, Ge, As, Se, Br, Rb, Sr, Y, Zr, Nb, Mo, Ag, Cd, In, Sn, Sb, I, Cs, Ba, La, Ce, Pr, Nd, Pb, Bi, Th, and U using a wide range of inorganic and organic certified reference materials. Phosphate rich standards NIST1400 (Bone Ash) and/or NIST1486 (Bone Meal) were used to check the calibrations. Further information on the PXRF method and its application in the St Andrews laboratory may be found in Stephens & Calder (2004), along with estimate of accuracy, precision and limits of detection.

The second batch was analysed using a SPECTRO XLAB 2000 with a 600W Pd anode tube, Si(Li) detector and the same five targets. The elements Al, Si, P, S, Cl, K, Ca, Ti, Mn, Fe, V, Cr, Ni, Cu, Zn, As, Se, Br, Sr, Zr, Cd, Sn, I, Ba and Pb were calibrated using the same range of standards. A selection of batch 1 samples was also run to ensure comparability between the datasets. The only heavy metals of significance not analysed for technical reasons in the second batch were Mo, U and Th. As most samples are present below the detection limit for these elements their absence in the second batch is not regarded as a significant omission.

3.4 Statistical analysis

The Minitab® (version 17) package was used for statistical analysis and graphical outputs. Differences between and within species in the levels of $\delta^{13}C$ and $\delta^{15}N$ in seabird guano and uric were analysed by using one-way ANOVAs, followed by a Tukeys post hoc test, although a pair t-test was used when there were less than two populations to compare. Differences in the levels of investigated trace elements were also analysed by using one-way ANOVAs, with a Tukey's post hoc test. A *p* value less than 0.05 with 95% Confidence Intervals (CIs) was taken to show a signficant statistical difference.

Chapter 4. Results

The aim of this chapter is to present the results for three main themes relating to the isotopic analysis of seabird guano and uric acid sampled from a diverse range of species and locations in Scotland between 2006 and 2009. This chapter also presents the findings of heavy metal analysis at seabird colonies over the same timescale.

4.1 Isotopic analysis of seabird guano

Over the study period, approximately 1000 samples were collected from a range of seabird species at breeding colonies around Scotland. A summary of the results from a subset of these samples is presented in Table 4.1.

4.1.1 Spatial variation in diet

The results show that the $\delta^{13}C$ and $\delta^{15}N$ values were highly variable across species and sampling locations in Scotland (Figure 4.1), which would be expected given the wide range of diets of the seabirds from which the guano samples were collected. However, there was evidence of dietary correlations in the same species at different geographical locations (Table 4.1).

The carbon ($\delta^{13}C$) values for guano ranged from -16.9 to -28.9‰ showing an obvious shift from a predominantly inshore water signature through to a diet dominated by terrestrial prey. The smallest variation in $\delta^{13}C$ was found in black-legged kittiwakes (*Rissa tridactyla)* from the Isle of May, while the greatest variation was found in Herring gulls (*Larus argentatus*) from Sanda and Ailsa Craig (-17.3‰ to -28.9‰ and -19.2‰ to -30.7‰ respectively). Figure 4.1 provides a presentational overview of a selection of the seabird species sampled during the study, showing patterns of obvious clumping by species as a result of foraging location.

In comparison, the nitrogen ($\delta^{15}N$) values of guano were less variable across species and sampling locations, ranging from 6.5‰ to 13.5‰. Some of the highest $\delta^{15}N$ values were found in Northern gannets (*Morus bassanus*) from Ailsa Craig and Bass

Table 4.1. A summary of the average and range of $\delta^{13}C$ and $\delta^{15}N$ values for guano samples collected at seabird colonies.

			$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)		Samples (n)
Location	Year	Species	Mean (+SD)	Range	Mean (+SD)	Range	
Foula	2006	Great skua	-18.4±1.39	-16.9 to -20.6	10.1±1.37	7.8 to 13.1	20
	2006	Arctic skua	-18.6±1.69	-16.0 to -20.6	8.5±0.91	7.0 to 10.1	12
	2006	Fulmar	-22.4±1.19	-20.9 to -23.8	10.5±1.59	9.2 to 13.5	12
	2006	European Shag	-16.9±0.77	-15.8 to -18.2	7.9±0.38	7.3 to 8.5	10
	2006	Razorbill	-20.5±0.37	-19.7 to -20.9	11.1±1.12	9.5 to 12.9	10
	2006	Kittiwake	-18.9±0.63	-17.3 to -20.3	8.4±0.60	7.4 to 9.3	13
	2006	Herring gull	-19.2±1.15	-17.8 to -20.6	9.6±0.55	9.0 to 10.5	15
Sanda	2006	Common gull	-25.09±4.21**[5]	-19.6 to -28.9	7.7±1.49	6.2 to 9.6	11
	2006	Herring gull	-25.2±4.11**	-17.3 to -28.9	8.8±1.63	5.2 to 10.7	16
Burnt Island	2006	Herring gull	23.1±2.21**	-17.9 to -26.3	9.4±0.64	8.3 to 10.7	20
Handa	2007	Arctic skua	-19.1±2.86	-16.5 to -23.5	10.6±1.45	8.1 to 12.2	8
	2007	Great skua	-16.9±1.62	-15.0 to -18.9	13.0±0.67	12.0 to 14.0	7
Ailsa Craig	2007	Northern Gannet	-19.6±0.64	-17.0 to -19.6	15.9±3.37	13.1 to 16.3	17
	2007	Lesser black-backed gull	-27.1±1.22	-25.7 to -28.7	8.6±1.39	7.1 to 11.1	8
	2007	Herring gull	26.7±2.51	-26.0 to -30.7	7.8±1.05	5.3 to 9.3	15
Rhum	2007	Manx shearwater	-22.3±1.32	-18.3 to -23.7	11.1±2.01	6.5 to 13.1	33
	2007	Black-legged kittiwake	-18.0±0.69	-17.3 to -19.3	9.1±0.53	8.5 to 9.6	7
St Kilda	2007	Great skua	-19.8±1.29	-18.7 to -22.0	10.4±1.36	7.7 to 12.2	10
	2008	Great skua	-17.5±0.48	-17.0 to 18.1	10.3±2.01	8.9 to 11.6	2
Bass Rock	2008	Northern Gannet	-20.4±0.55	-19.2 to -21.1	13.5±1.07	13.2 to 15.8	10
Isle of May	2007	Black-legged kittiwake	-18.9±0.98	-17.2 to -20.7	11.0±1.15	8.3 to 12.8	56
	2008	Black-legged kittiwake	-19.8±1.01	-18.2 to -21.7	10.6±0.52	9.9 to 11.8	25
	2009	Black-legged kittiwake	-19.6±1.09	-18.5 to 22.9	11.2±0.60	9.3 to 12.0	42
Dunbar harbour	2007	Black-legged kittiwake	-18.0±0.56	-17.0 to -18.9	10.8±0.55	9.9 to 12.9	32
	2008	Black-legged kittiwake	-19.5±0.77	-17.8 to 20.5	9.7±0.73	8.9 to 11.1	26
	2009	Black-legged kittiwake	-19.4±0.82	-17.4 to -21.1	9.3±1.54	7.1 to 12.0	25

[5] Carbon value means for some gull species are not an accurate representation of the data due to the diverse range of values (from a predominantly inshore to terrestrial diet).

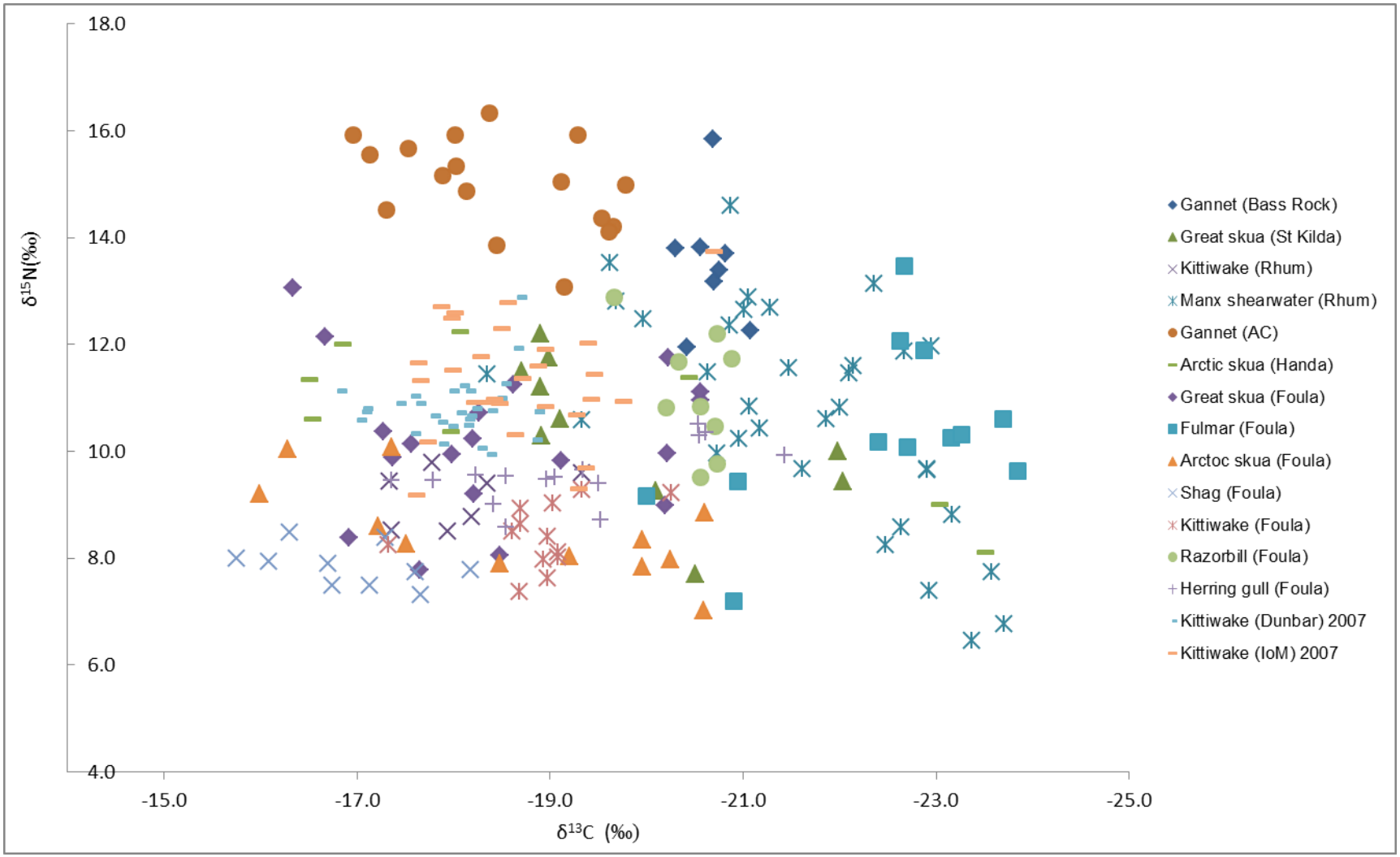


Figure 4.1. Range of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) ratios (‰) for guano concentrates from seabird species.

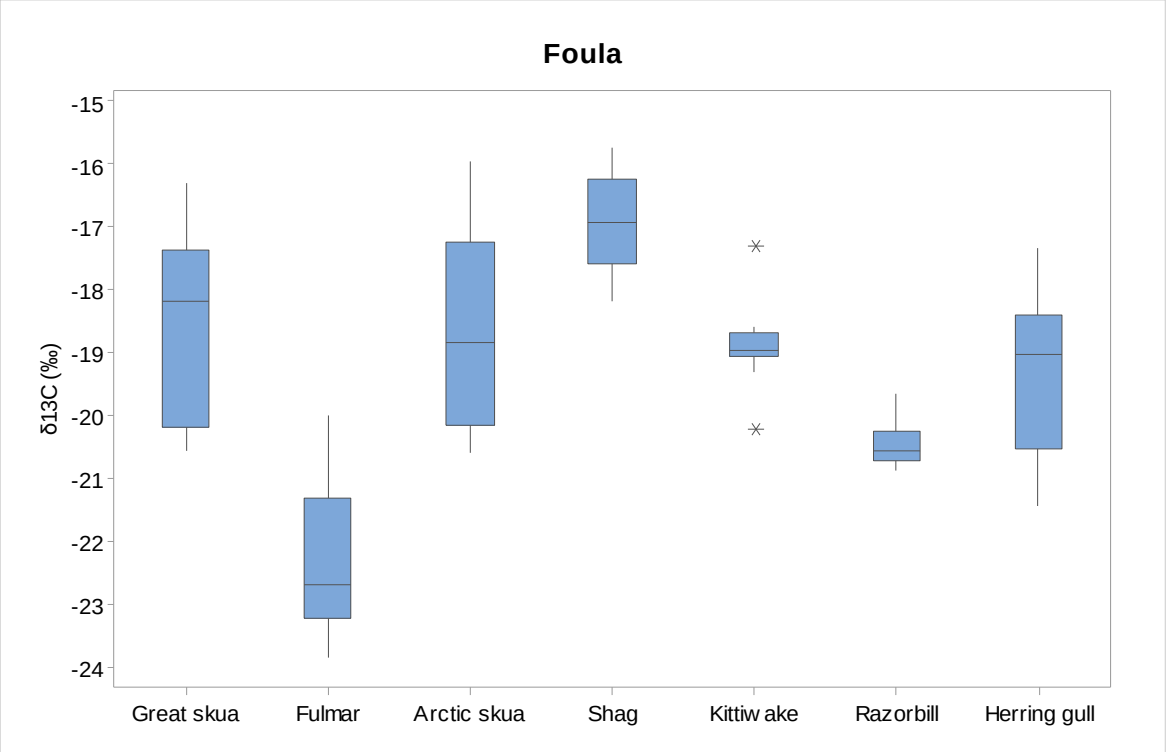
Rock (13.1‰ to 16.3‰) suggesting a diet at the higher trophic levels. European shags (*Phalacrocorax aristotelis*) and kittiwakes exhibited lower values, whilst Manx shearwaters (*Puffinus puffinus*) from the Isle of Rhum showed a surprisingly wide range of $\delta^{15}N$ values from 6.5‰ to 13.5‰ highlighting wide prey diversity (Figure 4.1 and table 4.1).

4.1.2 Interspecific variation

In most colonies there were patterns of interspecific variation in $\delta^{13}C$ and $\delta^{15}N$ signatures that would be expected given the species sampled. On both the Isle of Rhum and Ailsa Craig, there were significant differences between species sampled in each colony with no similarities in their $\delta^{13}C$ and $\delta^{15}N$ values; the only exception being herring gulls (*Larus argentatus*) and lesser black-backed gulls (*Larus fuscus*) on Ailsa Craig which had statistically similar values. Similarly, for gull species on Sanda, there was no significant variation in their $\delta^{13}C$ and $\delta^{15}N$ values. For species on Handa, there was no dietary difference between Great skuas (*Stercorarius skua*) and Arctic skuas (*Stercorarius parasiticus*) for $\delta^{13}C$ but significant differences for nitrogen with great skuas exhibiting higher $\delta^{15}N$ values.

Due to the greater diversity of species sampled on Foula, there was greater variation in the results. The $\delta^{13}C$ values of guano from Northern fulmars (*Fulmarus glacialis*) and shags showed significant differences to other species. Fulmars showed $\delta^{13}C$ values in the region of 3-4‰ lower than the other species sampled (mean value -22.4‰), characteristic of a diet heavily dominated by offshore prey. Shags in comparison exhibited much more enriched $\delta^{13}C$ values (mean value: -16.9‰), typical of an inshore diet. There was no significant difference between kittiwakes, great skuas, Arctic skuas and herring gulls (range: -18.4‰ to -19.2‰). In the case of observed $\delta^{15}N$ values, two distinct patterns of clumping were observed; the first in Razorbills (*Alca torda*), great skuas and fulmars (10.2‰ to 11.1‰) and the second at a lower trophic level in Arctic skuas, kittiwakes and shags (7.9‰ to 8.5‰). Figures 4.2(a) and (b) present box plots of the spread of $\delta^{13}C$ and $\delta^{15}N$ values for species from Foula. These patterns are consistent with the known diet of the species sampled,

(a)



(b)

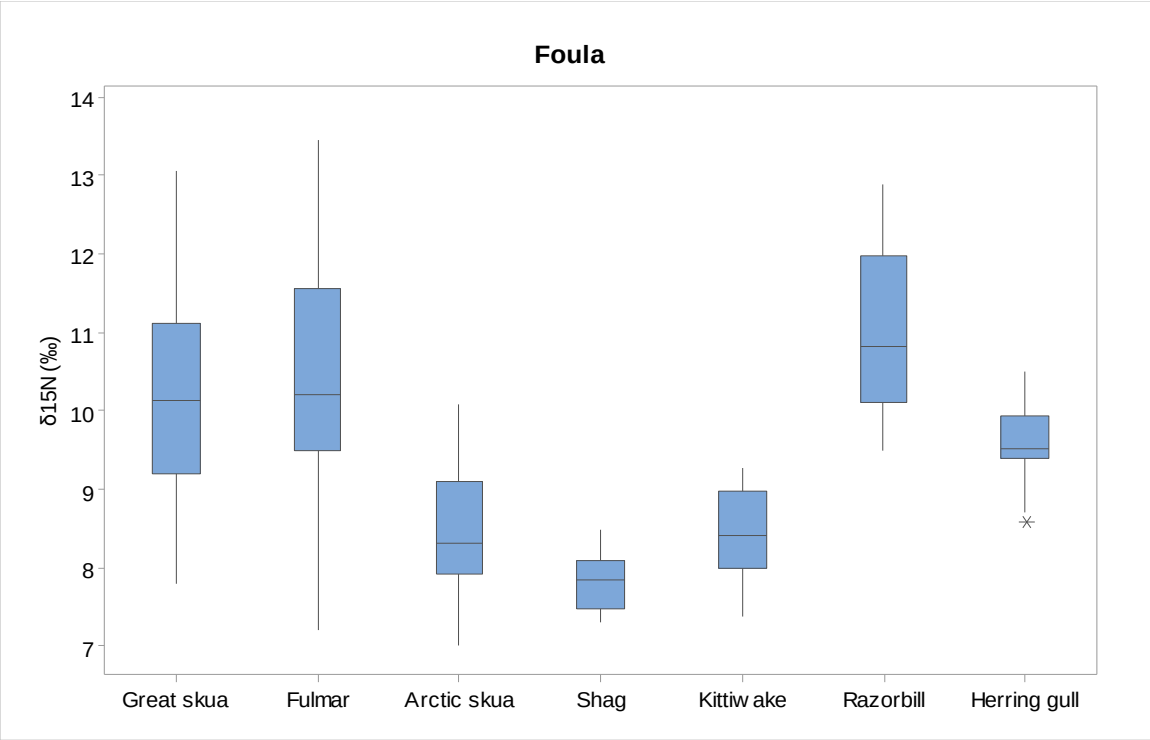


Figure 4.2 Box plots showing variation between seabird species on Foula for (a) $\delta^{13}C$ values and (b) $\delta^{15}N$ values in the guano of sampled species. The line represents the median values and the lines, 95% Confidence Intervals.

4.1.3 Geographical differences within species

The guano of Great skuas (*Stercorarius skua*), Northern gannets (*Morus bassanus*), Arctic skuas (*Stercorarius parasiticus*), Black-legged kittiwakes (*Rissa tridactyl*)[6] and Herring gulls (*Larus argentatus*) showed some significant differences among sampling locations for both $\delta^{13}C$ and $\delta^{15}N$ isotopes.

With respect to great skuas, birds from Handa showed significantly higher $\delta^{15}N$ values (12.0 to 13.4‰) than birds from St Kilda and Foula, suggesting that they are consuming larger or trophically higher prey. Furthermore, their $\delta^{13}C$ values were generally more enriched (2-3‰) than birds from other colonies, although this enrichment was only significant for birds from Handa and St Kilda. There were no significant differences between birds from St Kilda and Foula which showed more consistent values for both $\delta^{13}C$ and $\delta^{15}N$ values.

There were dietary differences between Northern gannets from Bass Rock and Ailsa Craig, with birds from Bass Rock showing slightly lower $\delta^{15}N$ values than birds from Ailsa Craig. In contrast, birds from Ailsa Craig were $\delta^{13}C$ enriched indicative of more inshore feeding habits. In both colonies the birds were feeding at a higher trophic level than the other sampled species

The $\delta^{13}C$ values for kittiwake guano were statistically similar for the Isle of May and Foula, whereas the Isle of May and Dunbar Harbour were statistically similar for $\delta^{15}N$. In contrast bird's samples from Foula showed significantly lower $\delta^{15}N$ values (up to 2‰ lower than the east coast colonies).

Herring gulls showed significant variation in $\delta^{13}C$ values between colonies characteristic of dietary diversity, with the greatest difference between Ailsa Craig and Foula (~6‰). There was less variation in $\delta^{15}N$ values across all colonies (Table 3.1), although significant differences were seen between sampling locations on the west, north and east coasts of Scotland, with each geographical area having significantly similar values.

[6] In the case of kittiwakes from the Isle of May and Dunbar Harbour, only values from the first year of sampling (2007) were used to allow for more appropriate comparisons.

Arctic skuas from Handa had a much wider range of $\delta^{13}C$ and $\delta^{15}N$ values than birds on Foula, with significantly higher mean $\delta^{15}N$ values (Table 4.1).

4.1.4 Temporal variation in diet of black-legged kittiwakes on the east coast of Scotland.

Between 2007 and 2009, samples were collected from kittiwakes at the Isle of May (Firth of Forth) and Dunbar Harbour (East Lothian) during the breeding season to provide a picture of within- and between-year variability. The results of these analyses are presented in Table 4.2.

Isle of May

Data from black-legged kittiwakes on the Isle of May showed that guano collected in 2007 there was more enriched in $\delta^{13}C$ (mean -18.8‰) compared to samples from 2008 and 2009 which were largely similar (means: -19.8‰ and -19.6‰ respectively). In contrast, $\delta^{15}N$ values were largely consistent between 2007 and 2008, whereas birds sampled in 2009 had significantly higher values (Table 4.2 and Figures 4.3 a & b).

Patterns of variation within sampled years were not consistent. In 2007 there were no significant differences in $\delta^{13}C$ values across the sampled months with a consistent spread of values. In contrast, average $\delta^{15}N$ values were significantly higher in June compared to the rest of the year (mean: 11.84‰). In 2009 there was no significant difference in nitrogen between months, while the $\delta^{13}C$ values for May were significantly different to July with a greater spread of values (Figures 4.3 a & b).

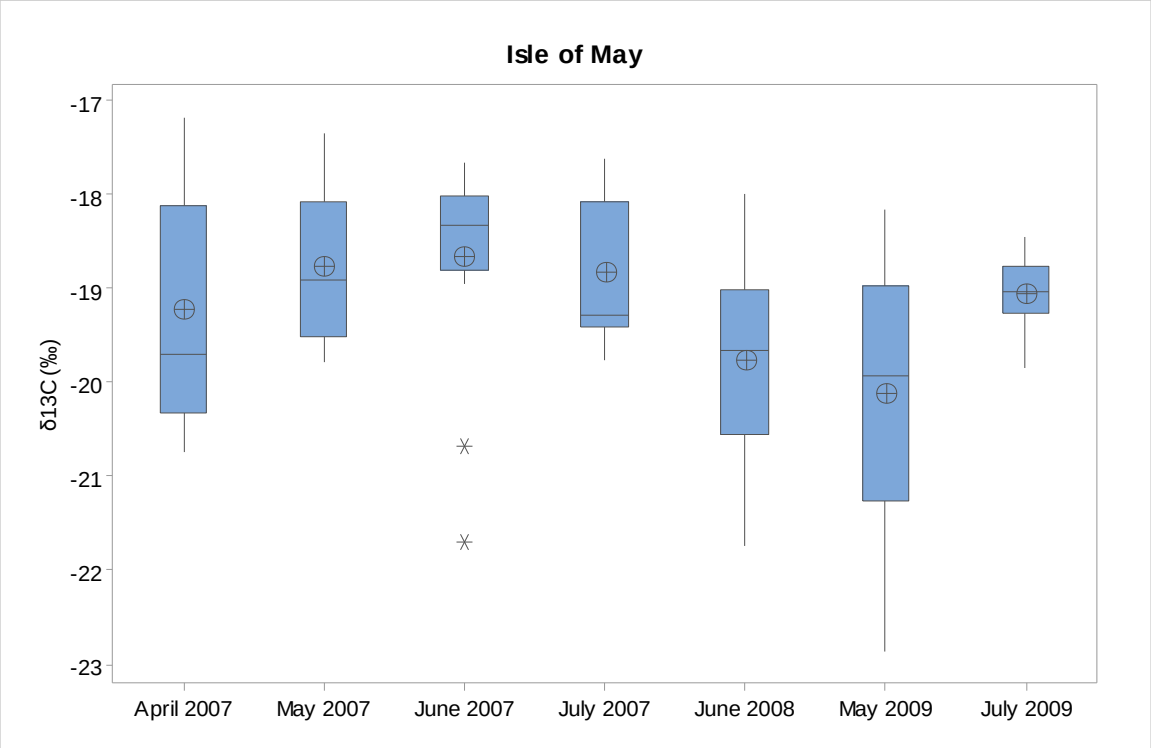
Dunbar Harbour

Similar to the Isle of May, guano collected in 2007 from Dunbar Harbour were more enriched in $\delta^{13}C$ (mean -18.04‰) compared to 2008 and 2009 which were largely similar (means: -19.53‰ and -19.40‰ respectively). A similar result was also found for nitrogen, with birds sampled in 2007 exhibiting significantly higher $\delta^{15}N$ values (mean – 10.8‰) than birds sampled in both 2008 and 2009 (means: 9.66‰ and 9.27‰ respectively) (Figures 4.4 a & b).

Table 4.2. A summary of the mean and range of $\delta^{13}C$ and $\delta^{15}N$ values for black-legged kittiwake samples collected between 2007 and 2009 from the Isle of May and Dunbar Harbour.

			$\delta^{13}C$ (‰)		**$\delta^{15}N$ (‰)**		
Location	**Year**	**Month**	**Mean (+SD)**	**Range**	**Mean (+SD)**	**Range**	**Samples (n)**
Isle of May	2007	April	-19.2±1.20	-17.2 to -20.7	10.9±1.51	8.3 to 12.8	14
		May	-18.9±0.78	-17.4 to -19.8	10.2±0.31	9.6 to 10.5	12
		June	-18.7±1.04	-17.7 to -20.7	11.8±0.85	10.3 to 12.3	17
		July	-18.8±0.77	-17.6 to -19.8	10.7±0.87	9.2 to 12.0	13
	2008	June	-19.8±1.01	-18.2 to -21.7	10.6±0.52	9.9 to 11.8	25
	2009	May	-20.1±1.31	-19.6 to -22.9	11.2±0.79	9.3 to 12.0	21
		July	-19.2±0.34	-18.5 to 19.8	11.4±0.32	10.8 to 11.8	21
Dunbar Harbour	2007	May	-18.0±0.47	-17.0 to -18.9	10.7±0.18	10.5 to 11.1	10
		June	-17.6±0.53	-16.8 to -18.6	11.0±0.42	10.3 to 11.9	11
		July	-18.5±0.33	-17.9 to -18.9	10.7±0.82	9.9 to 12.9	11
	2008	May	-19.1±1.00	-17.8 to 20.4	9.9±1.08	9.2 to 11.1	8
		June	-19.3±0.44	-18.7 to -20.1	9.7±0.42	9.0 to 10.3	8
		August	-20.1±0.45	-19.3 to -20.5	9.4±0.55	8.9 to 10.7	10
	2009	April	-20.0±0.59	-19.6 to -21.1	8.0±0.82	7.1 to 9.0	8
		May	-19.3±0.84	-17.4 to -19.9	8.8±0.54	8.0 to 9.3	10
		June	-19.3±0.71	-18.2 to -20.3	11.4±0.53	10.6 to 12.0	7

(a)



(b)

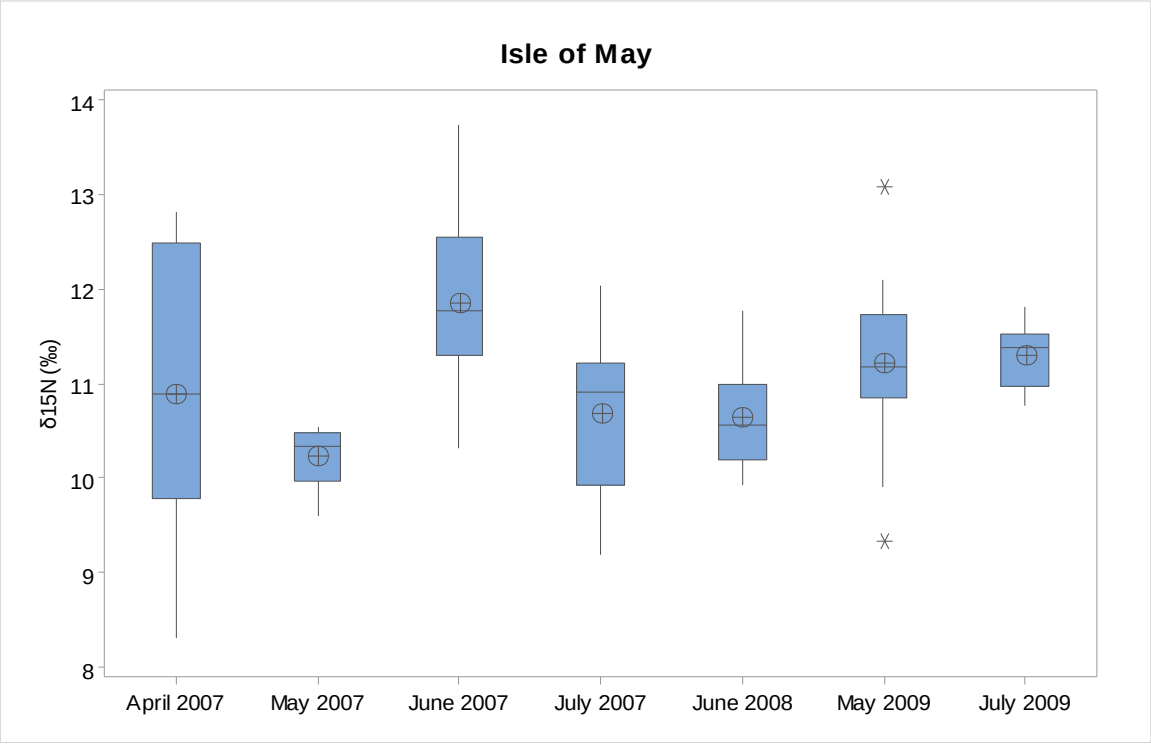
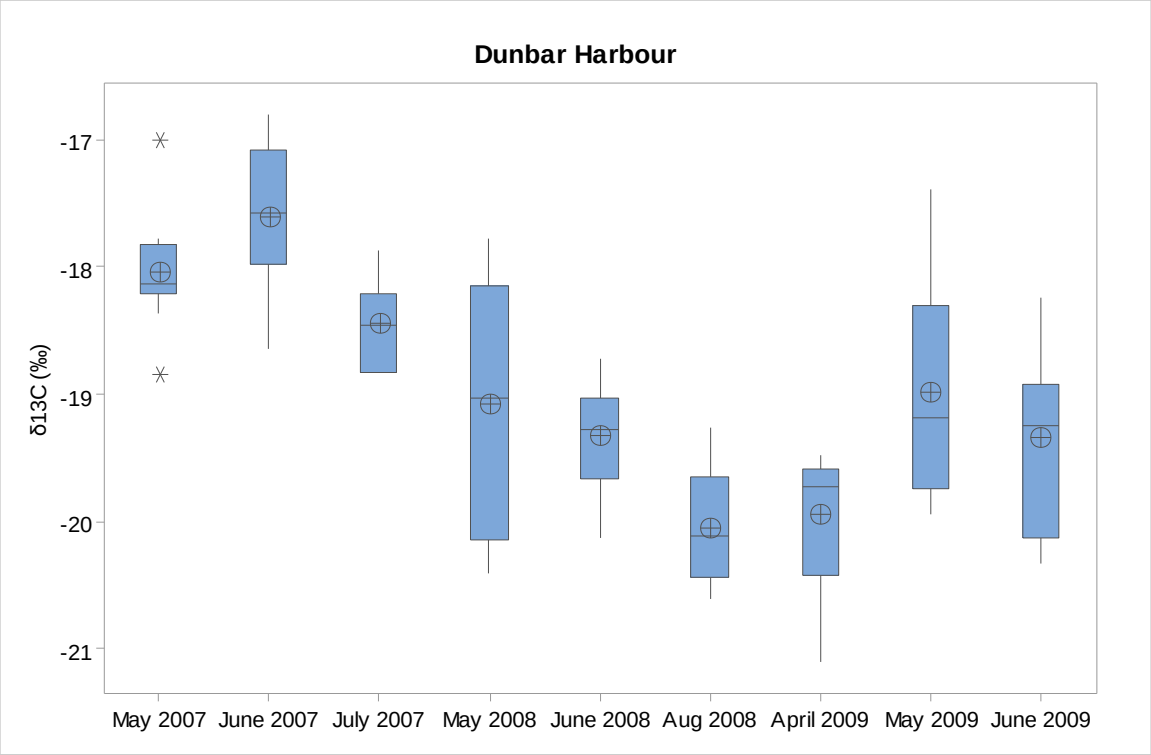


Figure 4.3. Box plots showing within- and between-year variation in guano samples from the Isle of May for a) $\delta^{13}C$, and b) $\delta^{15}N$ values. The line represents the median values and the lines, 95% Confidence Intervals.

(a)



(b)

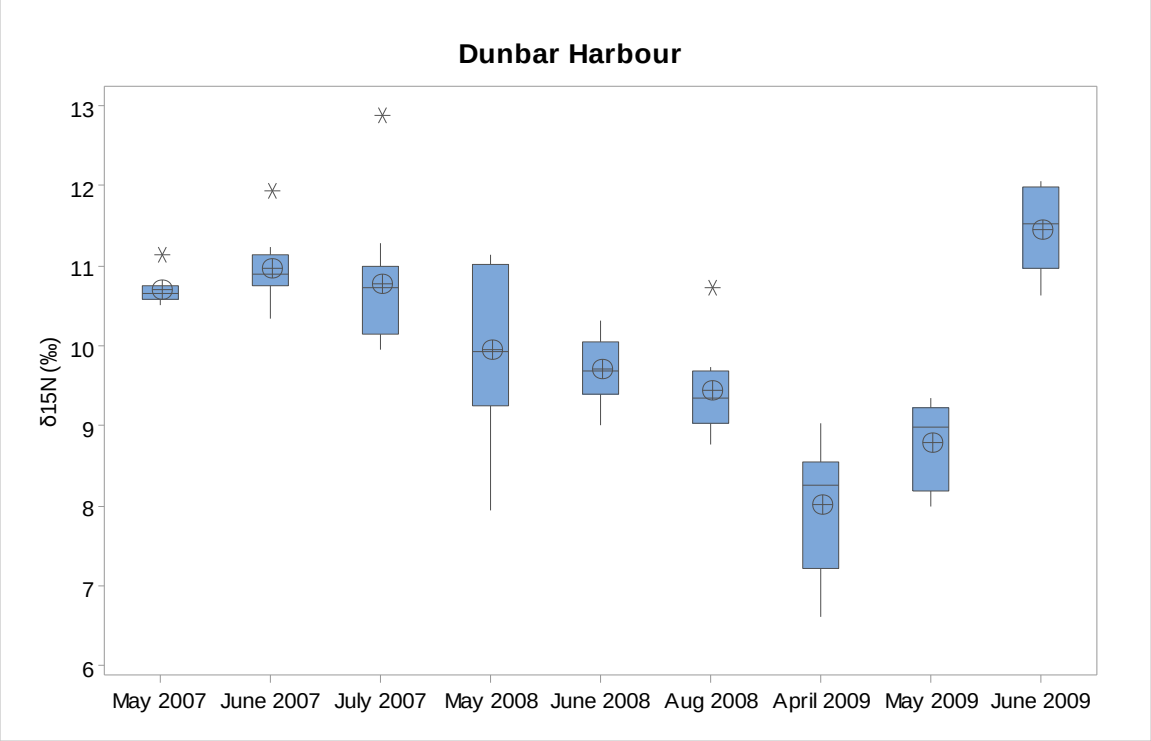


Figure 4.4. Box plots showing within and between year variation in guano samples from Dunbar Harbour for a) $\delta^{13}C$ and b) $\delta^{15}N$ values. The line represents the median values and the lines, 95% Confidence Intervals.

Compared to the Isle of May, guano samples taken from birds at Dunbar Harbour exhibited much lower within year variation (Figures 4.4 a & b). In 2007 and 2008, there were no significant differences in $\delta^{15}N$ values within years, although there was much wider variation in the standard deviation for June and July. In comparison, the $\delta^{15}N$ values of guano samples collected in June 2009 were significantly different to the rest of the year, with birds showing values up to 3.5‰ higher (11.43‰ in June, compared to 8.00‰ and 8.78‰ in April and May respectively).

With respect to $\delta^{13}C$ values, the samples showed greater within year variation. In 2007, there was no significant difference in $\delta^{13}C$ values within the year, with means largely consistent across months. Similarly, samples from 2008 and 2009 generally showed no significant differences in $\delta^{13}C$ values within years, with the exception of August 2008 and April 2009 where average $\delta^{13}C$ values were more depleted than the other months (although statistically similar to each other).

4.2 Isotopic analysis of uric acid (and relationship with seabird guano)

A summary of the results from guano samples that were subject to uric acid extraction are presented in Table 4.3 and Figure 4.5.

4.2.1 Spatial variation in diet using uric acid

Overall, the results show that like guano, the carbon and nitrogen values for uric acid varied considerably across species and sampling locations (Figure 4.5). However, there was evidence of some dietary similarities between species; a pattern also found in the guano signatures.

The $\delta^{13}C$ values for uric acid ranged from -15.2‰ to -28.9‰ showing a diversity of foraging locations from inshore to offshore waters as well as terrestrial environments. The smallest variation in $\delta^{13}C$ values was found in great skuas from St Kilda, while the greatest variation was found in herring gulls from Ailsa Craig. Figure 4.5 provides a presentational overview of uric acid signatures taken from a selection of the seabird species sampled during this study. Patterns of distinct clumping by species and location as a result of foraging behaviour are evident in the results.

Table 4.3. A summary of the average and range of $\delta^{13}C$ and $\delta^{15}N$ values for uric acid samples collected at seabird colonies.

			$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)		
Location	Year	Species	Mean (+SD)	Range	Mean (+SD)	Range	Sample (n)
Foula	2006	Great skua	-18.6±1.60	-16.1 to -20.7	9.2±0.93	7.9 to 11.1	15
	2006	Arctic skua	-18.4±1.77	-16.9 to -20.7	8.4±0.53	7.8 to 9.4	10
	2006	Fulmar	-22.6±1.54	-19.4 to -24.2	9.1±1.34	6.8 to 12.0	11
	2006	European Shag	-16.4±0.84	-15.6 to -18.4	7.6±0.56	6.4 to 8.1	12
	2006	Razorbill	-20.7±0.27	-20.2 to -21.0	9.4±0.34	9.0 to 10.1	9
	2006	Kittiwake	-19.1±0.80	-16.7 to -19.8	7.8±0.39	7.0 to 8.5	15
	2006	Herring gull	-19.0±0.76	-18.7 to -19.9	9.3±1.30	8.4 to 11.6	5
Sanda	2006	Common gull	-19.9±0.59**	-19.2 to -29.0	9.9±0.68	6.7 to 10.4	5
	2006	Herring gull	-17.9±1.79**	-16.3 to -26.9	10.1±0.43	8.3 to 10.6	4
Burnt Island	2006	Herring gull	-21.3±2.14**	-18.1 to -22.7	9.4±1.46	8.2 to 11.4	4
Handa	2007	Arctic skua	-16.5±0.31	-16.2 to -16.9	10.1±0.50	9.7 to 10.8	4
	2007	Great skua	-16.2±2.79	-14.5 to -19.4	12.2±0.25	11.9 to 12.4	3
Ailsa Craig	2007	Northern Gannet	-18.4±1.15	-16.8 to -20.1	11.8±1.49	8.6 to 13.1	8
	2007	Lesser black-backed gull	-23.3±1.07**	-22.5 to -24.0	10.1±0.63	9.7 to 10.6	6
	2007	Herring gull	-24.7±1.65**	-22.8 to -27.2	7.4±0.60	6.4 to 8.2	6
Rhum	2007	Manx shearwater	-22.2±1.60	-18.2 to -24.4	8.6±2.26	4.8 to 12.5	15
	2007	Black-legged kittiwake	-18.0±0.43	-17.6 to -18.5	10.3±1.13	9.1 to 11.4	3
St Kilda	2007	Great skua	-18.6±0.59	-18.0 to -19.4	9.5±1.60	7.2 to 11.1	5
	2008	Great skua	-18.3±1.37	-16.3 to -21.1	10.5±0.69	9.0 to 11.5	10
Bass Rock	2008	Northern Gannet	-18.6±0.99	-17.0 to -19.8	14.3±1.53	10.4 to 15.9	17
Isle of May	2007	Black-legged kittiwake	-18.5±1.10	-16.6 to -21.1	10.2±1.17	7.5 to 12.7	53
	2008	Black-legged kittiwake	-19.2±0.99	-17.6 to -22.0	10.1±0.85	8.2 to 11.5	19
	2009	Black-legged kittiwake	-19.2±1.07	-17.6 to -22.2	10.9±0.79	9.5 to 12.9	30
Dunbar harbour	2007	Black-legged kittiwake	-17.8±0.65	-16.7 to -19.0	10.5±0.47	9.8 to 11.9	21
	2008	Black-legged kittiwake	-19.3±0.84	-18.3 to -20.6	9.3±0.76	7.3 to 10.7	27
	2009	Black-legged kittiwake	-19.2±0.76	-18.1 to -20.5	9.4±1.90	6.9 to 12.1	15

** Carbon value means for some gull species are not an accurate representation of the data due to the diverse range of values (from a predominantly inshore to terrestrial diet).

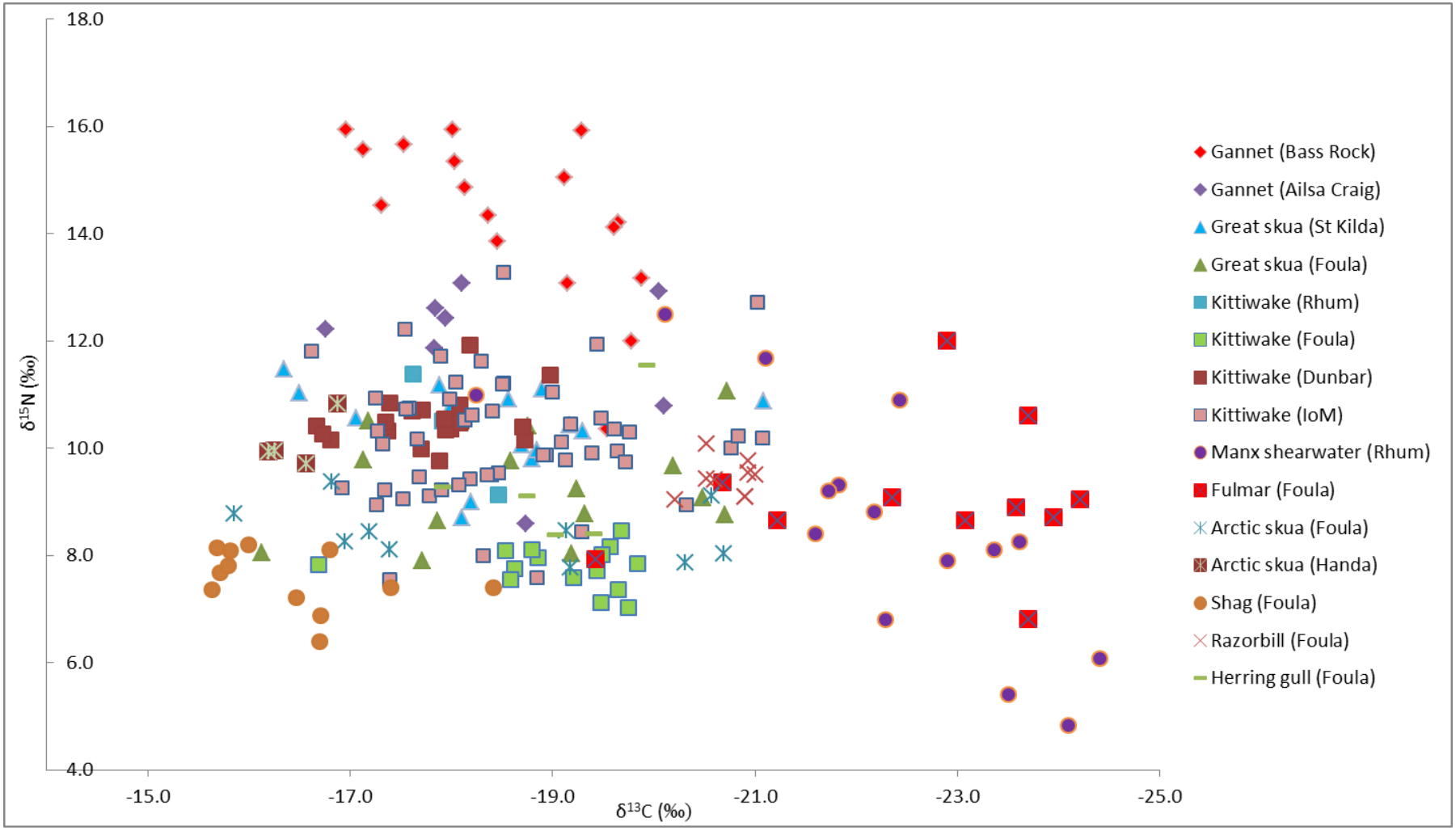


Figure 4.5. Carbon ($\delta^{13}C$) and nitrogen isotope ($\delta^{15}N$) results for uric acid concentrates derived from a range of seabird species from colonies around the Scottish coast.

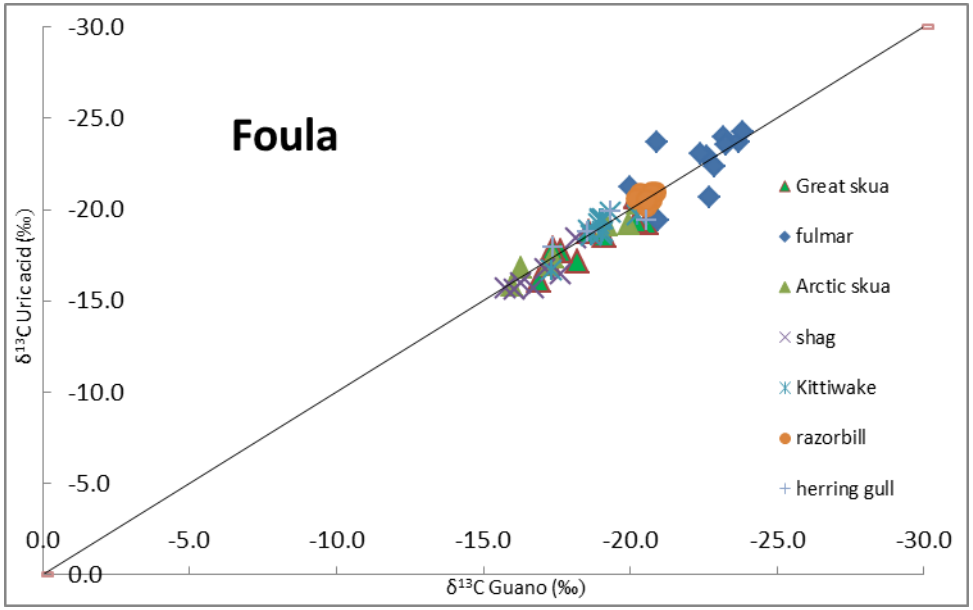
The $\delta^{15}N$ values for extracted uric acid ranged from 5.2‰ to 16.3‰, showing great diversity in dietary habits across species and locations. As was found in bulk guano, the highest values were found in Northern gannets from Bass Rock and Ailsa Craig (16.3‰ and 15.8‰ respectively). Similar to guano, some of the lowest levels were found in shags from Foula (mean: 16.9‰), whilst Manx shearwaters from the Isle of Rhum showed the greatest range in $\delta^{15}N$ values (4.8‰ to 12.5 ‰). These results were generally consistent with the isotopic composition of guano from a selection of the same samples.

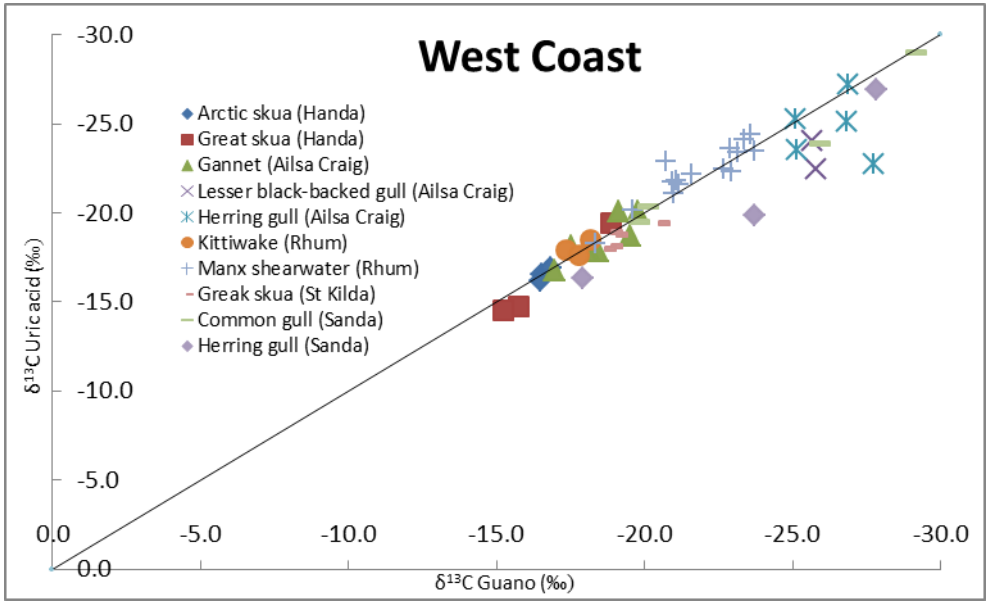
4.2.1 Relationship between the stable isotopic composition of seabird guano and extracted uric acid

Figures 4.6 (a-e) and 4.7 (a-e) present the findings of the relationship between the carbon and nitrogen composition of guano samples from a range of seabird species and the uric acid concentrations from the same samples. For presentational ease, the samples are separated into West coast (e.g., St Kilda, Handa, Ailsa Craig and Sanda), East coast (e.g., Bass Rock and Burnt Island) and Foula (North coast). Dunbar Harbour and the Isle of May were considered separately due to the time series of data available from the sites (breeding season 2007 to 2009); all which were considered in this analyses.

In all cases, the $\delta^{13}C$ values of uric acid were not significantly different from that of guano with an average difference of <0.85‰ when the complete series of samples were considered in their entirety. Figure 4.6 (a-e) presents this highly linear relationship between guano and uric acid when scattered around a 1.1 line. Some gull species from Ailsa Craig (e.g., Lesser black-backed gulls and herring gulls) showed more variation between guano and uric acid with differences up to 2.5‰, but this was still considered not statistically significant Figure 4.6b).

In comparison, the relationship between the $\delta^{15}N$ values for guano and uric acid shows some differences, with the samples generally sitting on or below the 1.1 line (Figure 3.7 (a-e)). In all seabird species, the $\delta^{15}N$ values of uric acid were not significantly different from that of guano for the same sample, with an average difference of <1.2‰. As was the case with the results from the $\delta^{13}C$ analyses,





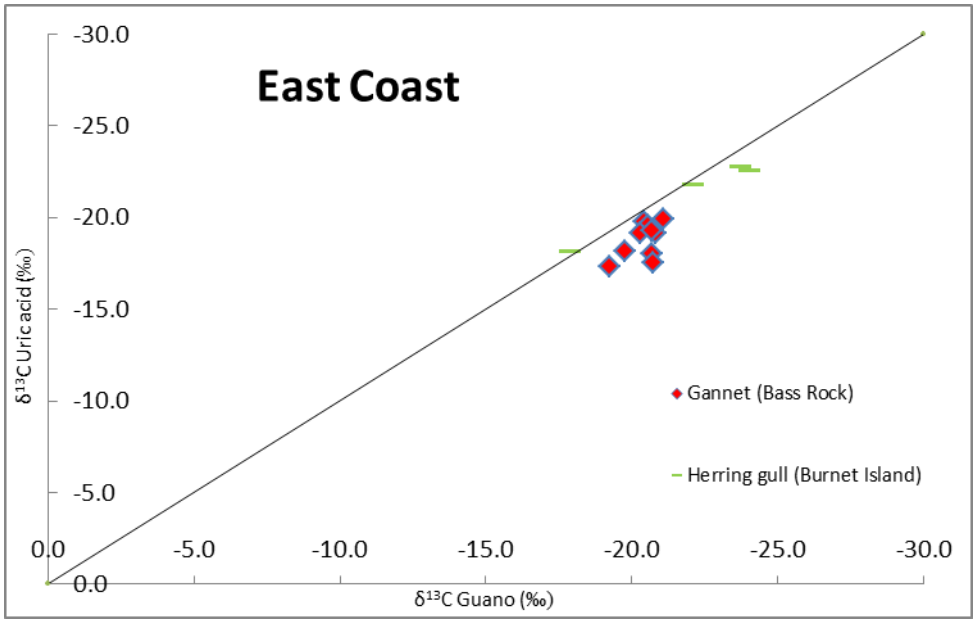
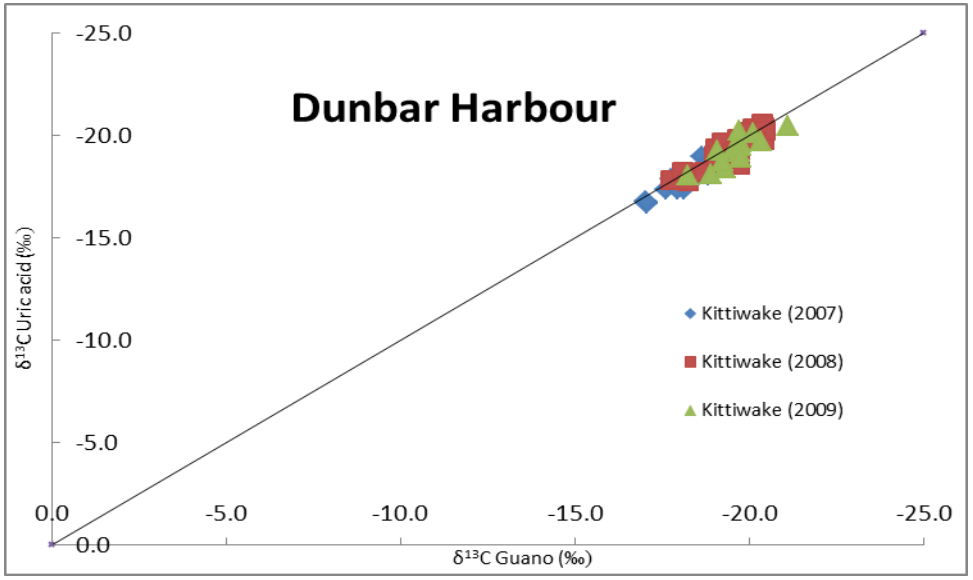
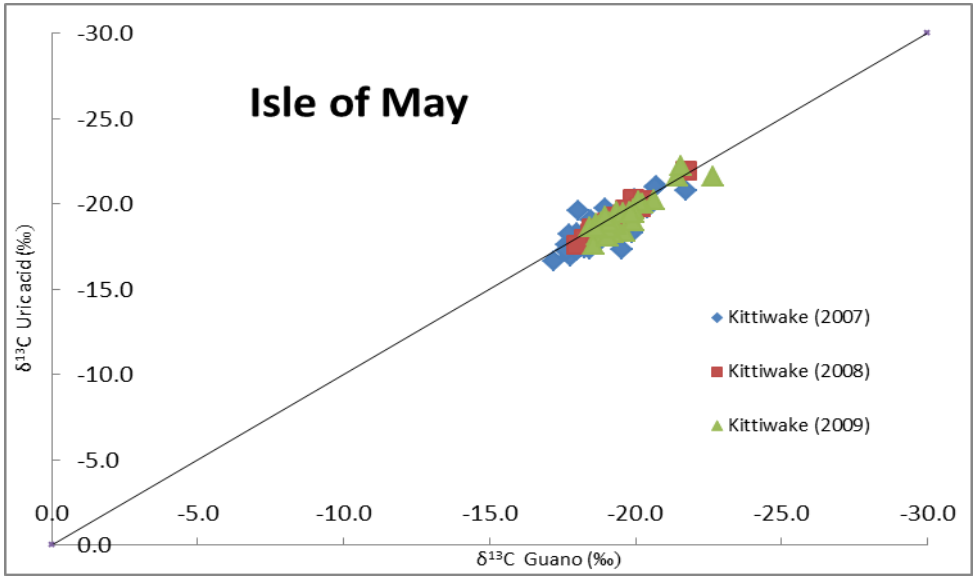
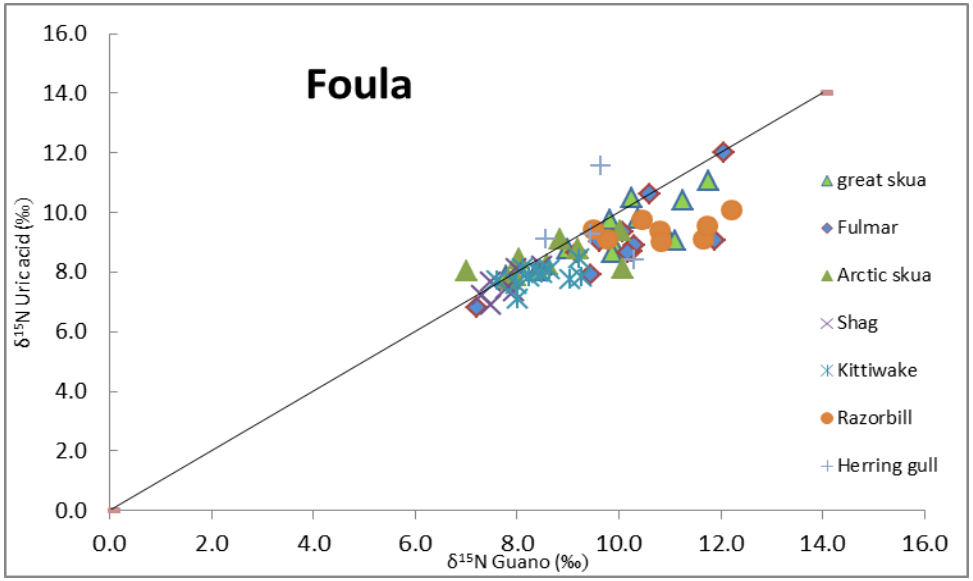
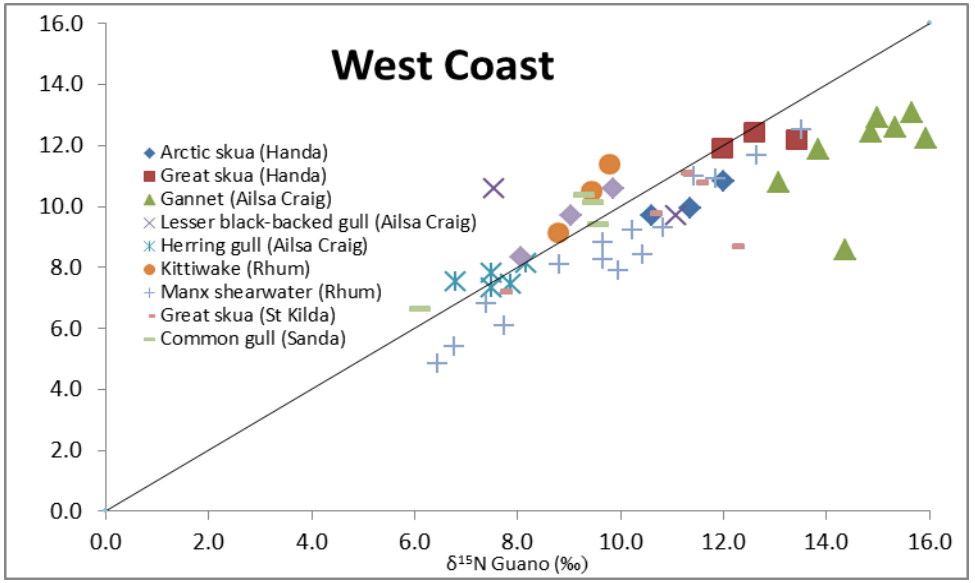


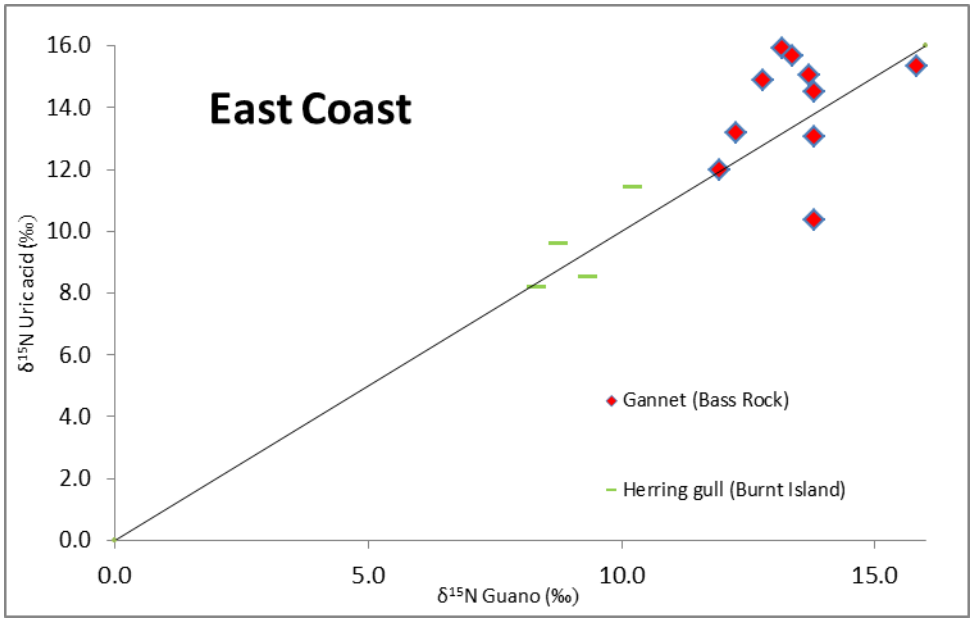
Figure 4.6. Relationship between the carbon isotopic composition of guano from a range of seabird samples and uric acid concentrations from the same samples for the colonies on (a) Foula; (b) west coast, (c) east coast, (d) Dunbar Harbour and (e) Isle of May. Error bars on the individual analyses are smaller than the symbols.

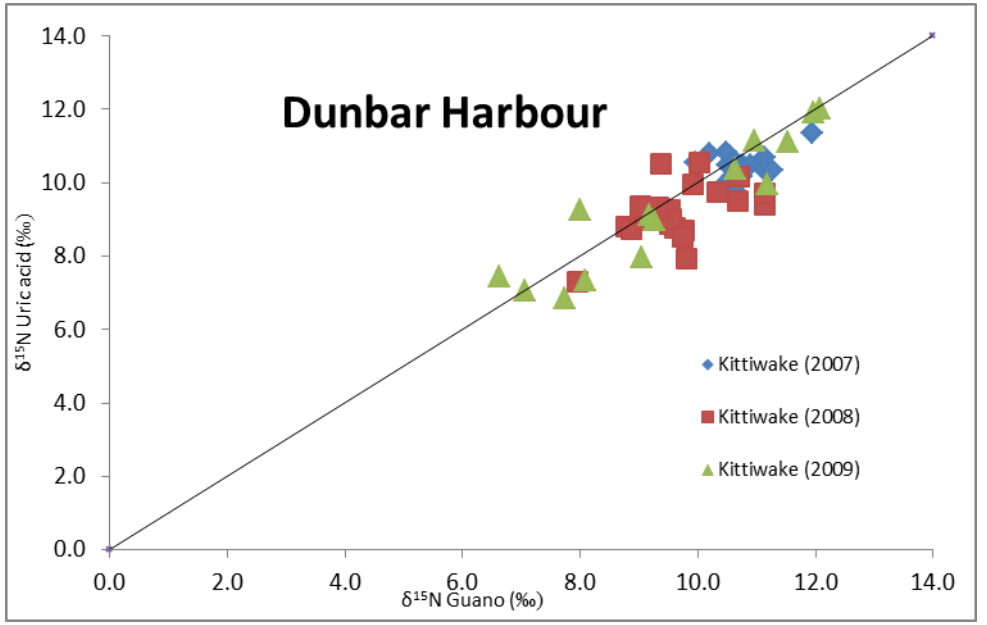

Dunbar Harbour
-25.0
-20.0
-15.0
-10.0
-5.0
0.0
-5.0
-10.0
-15.0
-20.0
-25.0
0.0
δ13C Uric acid (‰)
δ13C Guano (‰)
Kittiwake (2007)
Kittiwake (2008)
Kittiwake (2009)


Isle of May
-30.0
-25.0
-20.0
-15.0
-10.0
-5.0
0.0
-5.0
-10.0
-15.0
-20.0
-25.0
-30.0
0.0
δ13C Uric acid (‰)
δ13C Guano (‰)
Kittiwake (2007)
Kittiwake (2008)
Kittiwake (2009)


Foula
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
δ15N Uric acid (‰)
δ15N Guano (‰)
great skua
Fulmar
Arctic skua
Shag
Kittiwake
Razorbill
Herring gull


West Coast
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
δ15N Guano (‰)
Arctic skua (Handa)
Great skua (Handa)
Gannet (Ailsa Craig)
Lesser black-backed gull (Ailsa Craig)
Herring gull (Ailsa Craig)
Kittiwake (Rhum)
Manx shearwater (Rhum)
Great skua (St Kilda)
Common gull (Sanda)





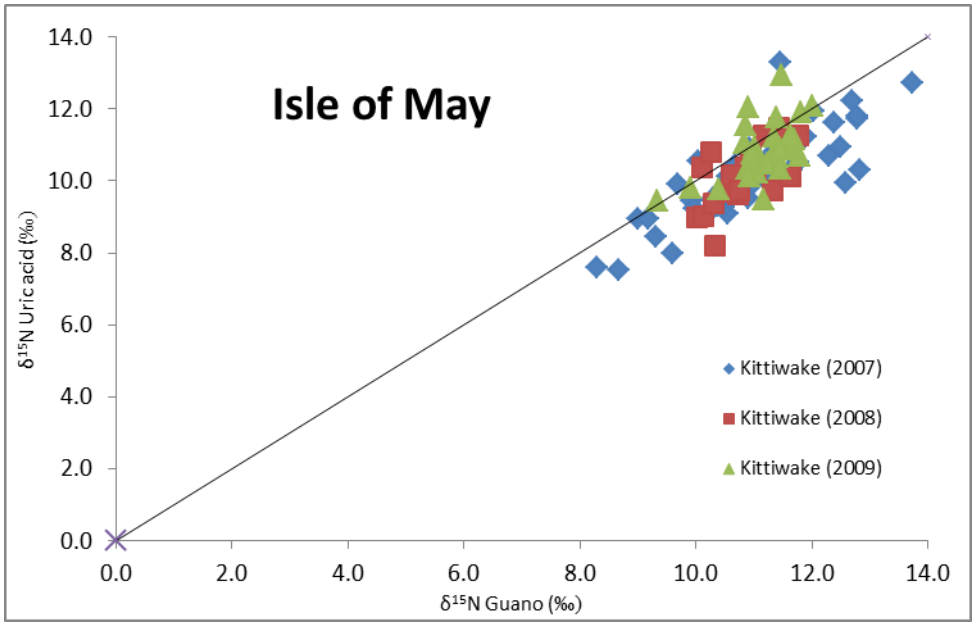


Figure 4.7. Relationship between the nitrogen isotopic composition of guano from a range of seabird samples and uric acid concentrations from the same samples for the colonies on (a) Foula; (b) west coast, (c) east coast, (d) Dunbar Harbour and (e) Isle of May. Error bars on the individual analyses are smaller than the symbols.

some samples (e.g., kittiwakes and razorbills from Foula, gannets from Ailsa Craig and kittiwakes from the Isle of May), did show uric acid $\delta^{15}N$ values up to 2.5‰ lower than the guano from the same sample (Figure 4.7 a, b and e) but this was not considered significantly different. In the case of kittiwakes from the Isle of May, the difference between guano and uric acid were only seen in samples collected in 2007 and 2008; values for 2009 showed no difference between uric acid and guano $\delta^{15}N$ values for the same sample. Gannets from Bass Rock also showed differences in the case of higher $\delta^{15}N$ values for uric acid, but these were within the significance range so not considered statistically different.

4.4 Heavy metal concentrations in breeding seabirds

A summary of the concentrations of Mn, Fe, Cu, Zn, As, Se, Cr, Br, Zn, Cd and Pb in the guano of a selection of seabird species from around Scotland are presented in Table 4.4. Other trace elements (e.g., Ag, Hg and Sn) were not considered as their detection limit was too low.

4.4.1 Intraspecific difference

Samples from species across multiple locations were collected for kittiwakes (e.g., Dunbar Harbour, Isle of May and Foula), great skuas (e.g., Handa, St Kilda and Foula) and shags (e.g., Handa and Canna), allowing geographical differences within species to be observed. In the case of kittiwakes from the Isle of May and Dunbar Harbour, data from 2007 was only considered for this analysis of intraspecific differences.

Black-legged kittiwake

The highest levels of Mn, Fe, Cu and Zn were detected in the guano of birds from Dunbar Harbour, whereas the guano of kittiwakes from the Isle of May showed the highest levels of Cr and Pb respectively (Table 4.4). Kittiwakes from Foula showed higher Sr levels than birds at the other locations, but significantly lower levels of Cu and Zn. There was no significant difference between birds from the three locations for As and Br.

Table 4.4. Metal and trace element concentrations (ppm) in guano of seabirds from different locations around Scotland. Data shown are means ± standard deviation (n: number of samples).

Species	Mn	Fe	Cu	Zn	As	Se	Cr	Pb	Sr
Black-legged kittiwake									
Dunbar Harbour[1] (n=54)	29.24±19.84	1189.0±959.4	16.39±9.67	317.65±99.60	7.82±18.18	3.94±1.14	10.09±4.50	7.79±5.43	378.2±305.4
Isle of May[1] (n=40)	5.13±2.72	146.48±160.98	8.10±5.08	242.27±108.59	18.41±73.22	4.09±1.07	3.98±4.82	12.84±8.51	377.31±305.8
Foula (n=10)	19.20±8.49	202.86±75.42	5.07±1.45	106.46±14.17	3.59±1.04	4.44±0.49	5.42±1.93	11.52±1.52	394.2±48.7
Great skua									
Foula (n=10)	4.09±1.87	27.27±30.41	4.34±2.12	78.80±45.73	1.53±0.61	2.55±0.83	5.35±0.64	7.79±3.22	281.4±109.6
St Kilda (n=5)	4.74±1.47	133.52±56.35	15.14±6.99	131.86±25.24	2.18±0.43	3.60±1.13	**	9.88±1.99	590.7±210.2
Handa (n=2)	7.95±3.89	107.5±105.35	14.5±6.22	131.15±19.30	2.00±0.42	4.5±0.99	**	10.55±2.47	841.8±349.7
Shag									
Foula (n=12)	16.17±10.53	52.88±25.56	7.70±3.63	175.63±72.38	2.97±1.97	4.60±0.92	**	12.87±3.12	502.1±169.6
Canna (n=4)	11.23±1.65	62.28±18.43	8.20±2.54	146.15±17.95	1.33±0.45	3.45±0.70	**	7.55±2.48	517.2±133.3
Herring gull									
Sanda (n=5)	76.90±13.06	2708.20±852.57	16.38±2.15	245.96±27.35	7.50±4.47	5.72±1.02	40.9±11.78	36.56±10.63	385.5±109.8
Gannet									
Bass Rock (n=5)	5.84±3.33	1045.24±309.31	35.86±9.00	210.80±23.60	8.06±2.56	8.60±1.81	5.22±3.02	40.34±13.12	359.8±190.6
Manx shearwater									
Rhum (n=2)	5.60±0.56	212.25±54.94	15.05±10.25	87.70±26.59	<1	1.35±0.50	24.80±7.78	4.40±0.14	152.5±10.3

** Samples are below the detection limit. [1] The mean concentrations provided for kittiwakes from the Isle of May and Dunbar in the table are summed for 2007 to 2009. However, when testing for statistical signification between geographical locations, only samples from 2007 were considered due to the inter-annual variation in some elements.

Great skua

For great skuas, the highest levels of Fe, Cu, Zn and As were detected in the guano of birds from St Kilda, whereas the birds from Handa showed the highest levels of Mn, Se and Pb in their guano. There was no significant difference between the three locations for Mn, Zn, As, Pb, Cr and Cd, whereas great skuas from Foula had significantly lower levels of Cu, Se, Br and Sr and Fe than the other colonies studied.

European shag

European shags from Foula showed the highest levels of Mn, Zn, As and Pb in their guano, whereas the guano of birds from Canna had the highest levels of Fe and Cu. In most cases there was no significance difference between the two locations, with the exception of Pb, Zn, As and Se where birds from Foula had significantly higher levels in their guano.

4.4.2 Interspecific differences

Analyses were undertaken to establish differences in the levels of trace elements across species. Consideration was given to species at a single colony (e.g., Foula) and across geographical boundaries (e.g., east, west and north coasts of Scotland). Figure 4.8 (a) to (k) present the findings of this across species analyses for all trace elements studied.

Foula

On Foula, the guano of kittiwakes, great skuas and shags showed significant differences for several trace elements. The highest levels of Mn, Fe, As and Cr were detected in the guano of kittiwakes, whereas the guano of shags showed the highest levels of Cu, Zn, Se and Pb. Great skua guano showed significantly lower levels of Mn, Cu, As, Sr , Se and Pb in comparison to the guano of kittiwakes and shags, although across all trace elements analysed, great skuas consistently had the lowest levels of all three species.

Across geographical colonies

As would be expected, there was significant variation in the levels of trace elements across species. Each trace element is considered in turn:

Manganese: Herring gulls from Sanda had significantly higher levels (76.9±13.1ppm) than any of the other sampled species, although kittiwakes from Dunbar also exhibited relatively high levels (29.24±19.84 ppm). The rest of the species all had statistically similar levels (mean value<19.20ppm) with very little variation in guano Mn levels (Figure 4.8a).

Iron: Again, Herring gulls from Sanda had significantly higher levels (2708±853ppm), although kittiwakes from Dunbar (2007) and gannet from Bass Rock also had elevated levels (1187±1093 and 1045±309ppm respectively). The rest of the species were statistically similar with mean Fe levels <231ppm. There was considerable variation within the samples of some species (Figure 4.8b).

Copper: Gannets from Bass Rock had significantly higher levels (35.9±9.01ppm) than other species. The rest of the species sampled had statistically similar levels (mean ranging from 16.4 to 8.2ppm), with the exception of all species from Foula which had the lowest levels (mean<7.7ppm). Manx shearwaters (Rhum) and great skuas (St Kilda) showed the great variation of Cu in the samples (Figure 4.8c).

Zinc: Most species sampled had statistically similar levels (means ranging between 175.6 and 78.8ppm) in their guano. Kittiwakes from Dunbar and the Isle of May from 2007 and herring gulls from Sanda had higher mean levels >246ppm, while great skuas from Foula had the lowest (78.8±45.7ppm). There was little variation in the samples of most species, with the exception of kittiwakes from the Isle of May and skuas from Foula (Figure 4.8d).

Arsenic: There was no significant difference between the mean values for As across the guano samples (mean <9ppm) whereas the level of variation within each species was high. The highest levels of As were found in kittiwakes from the Isle of May in 2007 (10.41±12.6ppm) and the lowest in Manx shearwaters from Rhum (1±0.0ppm). The majority of sampled species had mean levels<3.5ppm Figure 4.8e).

Selenium: Guano samples from Bass Rock gannets had significantly higher Se levels (8.6±1.81ppm) than the other species. The rest of the samples were generally

consistent (with mean values between 5.7 and 3.6ppm), with the exception of kittiwakes from Dunbar harbour, great skuas from Foula and Manx shearwaters from Rhum which all had low levels (mean values of 1.3 to 3.2ppm). There was very little variation within the samples for each species (Figure 4.8f).

Chromium: Herring gulls from Sanda had significantly higher levels (40.9±11.7ppm) of Cr, although Manx shearwaters from Rhum also had significantly elevated levels (24.8±7.9ppm). Levels in guano from the rest of the sampled species were not significantly different (Figure 4.8g).

Lead: Guano from gannets (Bass Rock) and herring gulls (Sanda) had significantly higher levels of Pb (40.34±13.1ppm and 36.56±10.6ppm respectively) than the other species sampled. Guano from the rest of the species had statistically similar Pb levels, although Manx shearwaters from Rhum did have the lowest mean levels (4.4±0.1ppm) (Figure 4.8h).

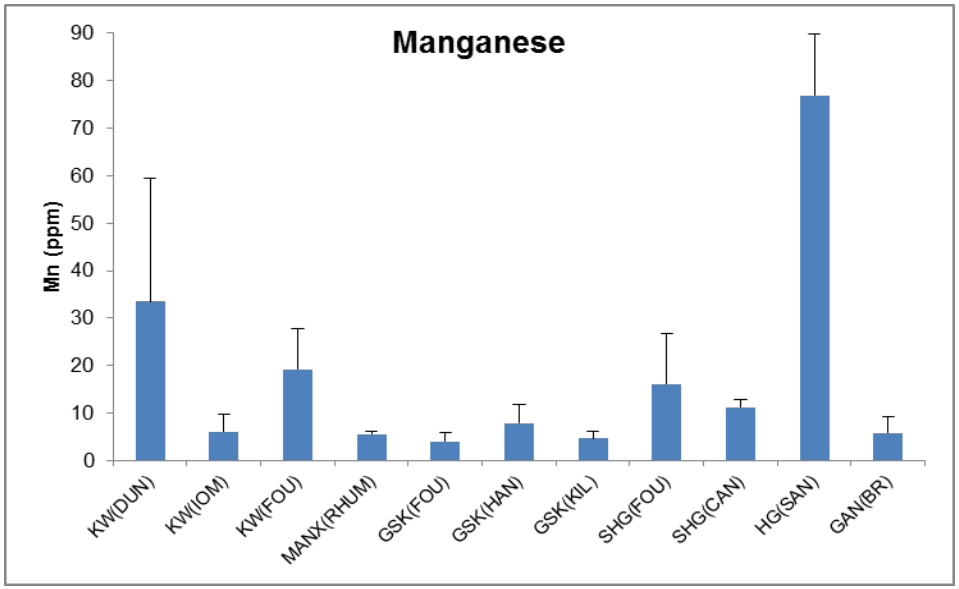
Strontium: Great skuas from Handa had the highest levels (mean: 842±133ppm), although they were not significantly different from great skuas (St Kilda) and shags (Foula and Canna) which also had elevated levels (mean: 502 to 590ppm). Manx shearwaters from Rhum had the lowest mean level and subsequent sample variation (152.5±10.3ppm) (Figure 4.8i).

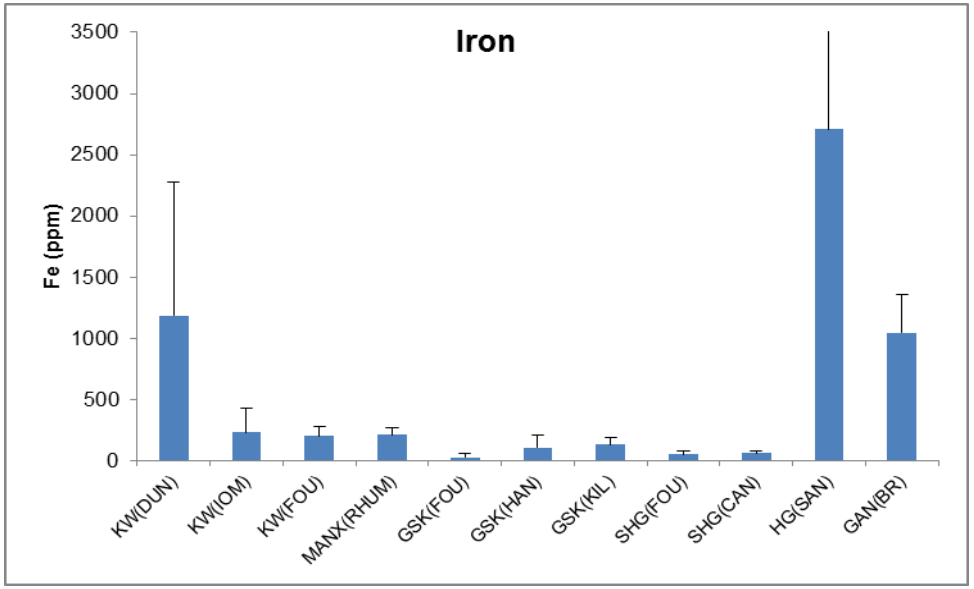
4.4.3 Temporal differences - Intraspecific differences within black-legged kittiwakes on the east coast of Scotland

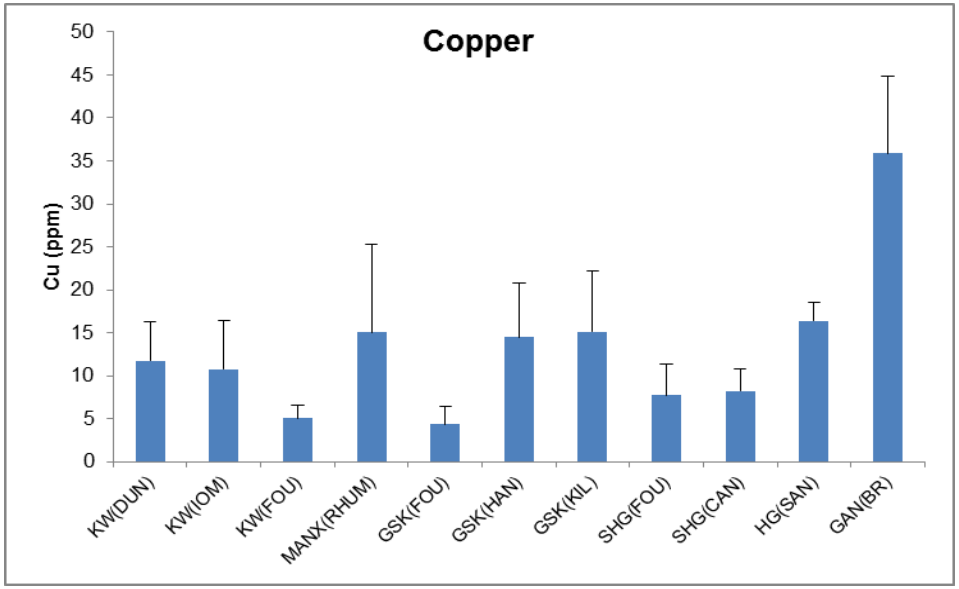
Between 2007 and 2009, guano samples collected from kittiwakes on the Isle of May (Firth of Forth) and at Dunbar Harbour (East Lothian) during the breeding season were analysed for trace elements to provide a picture of within and between year variability.

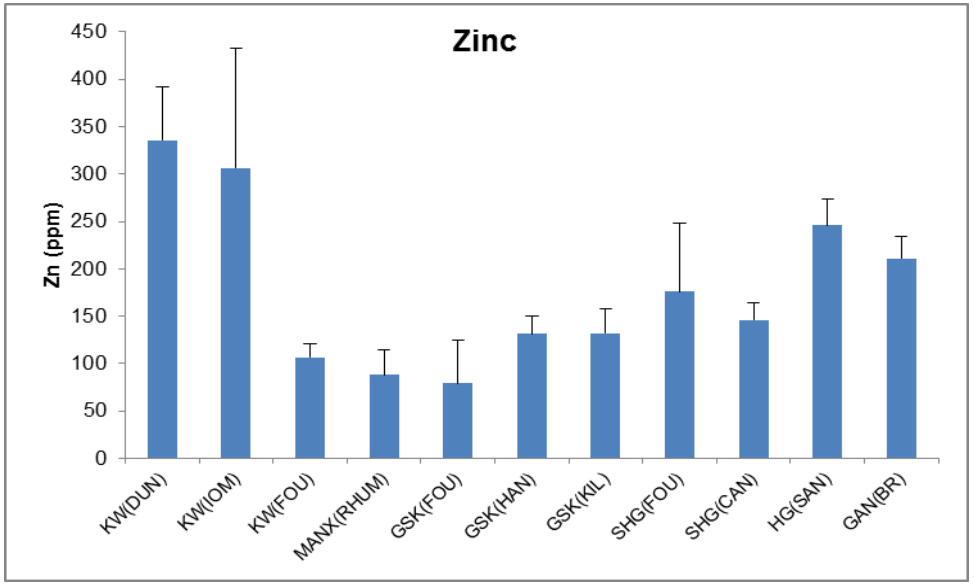
Isle of May

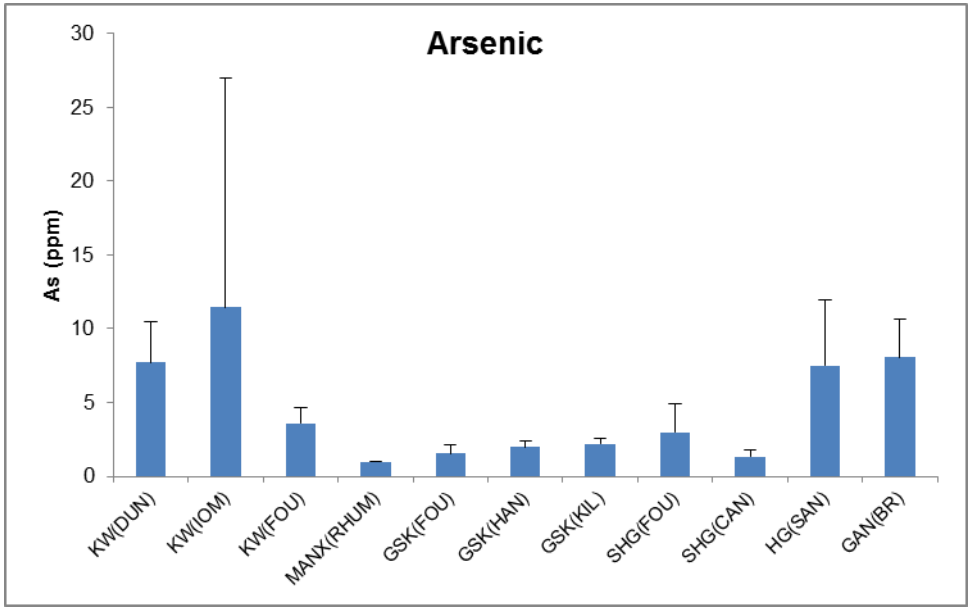
Kittiwake guano from the Isle of May showed no significant difference in Mn, As, Se and Pb between the sampling years (2007 to 2009). Samples from 2007 had

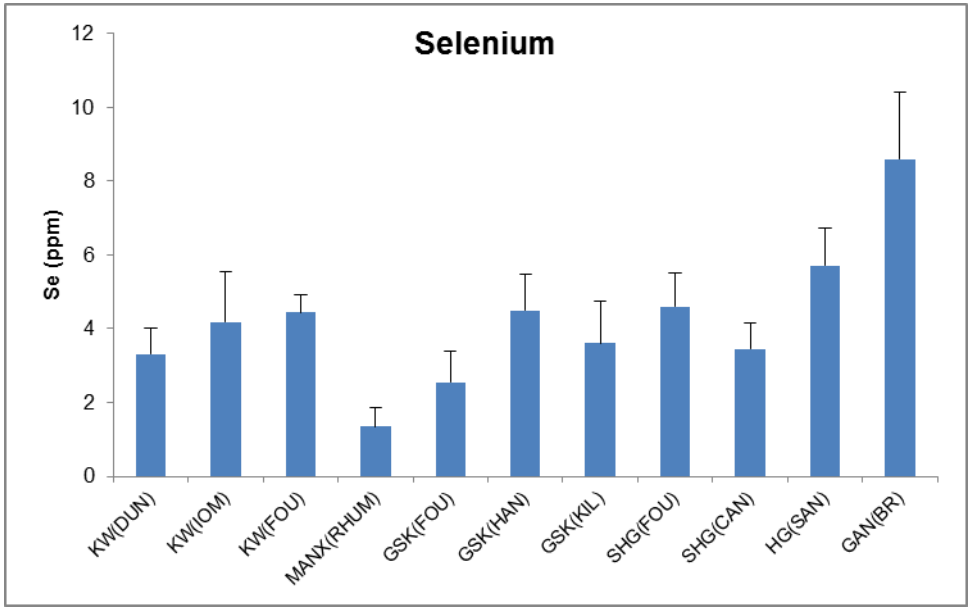

Manganese
Mn (ppm)
90
80
70
60
50
40
30
20
10
0
KW(DUN)
KW(IOM)
KW(FOU)
MANX(RHUM)
GSK(FOU)
GSK(HAN)
GSK(KIL)
SHG(FOU)
SHG(CAN)
HG(SAN)
GAN(BR)

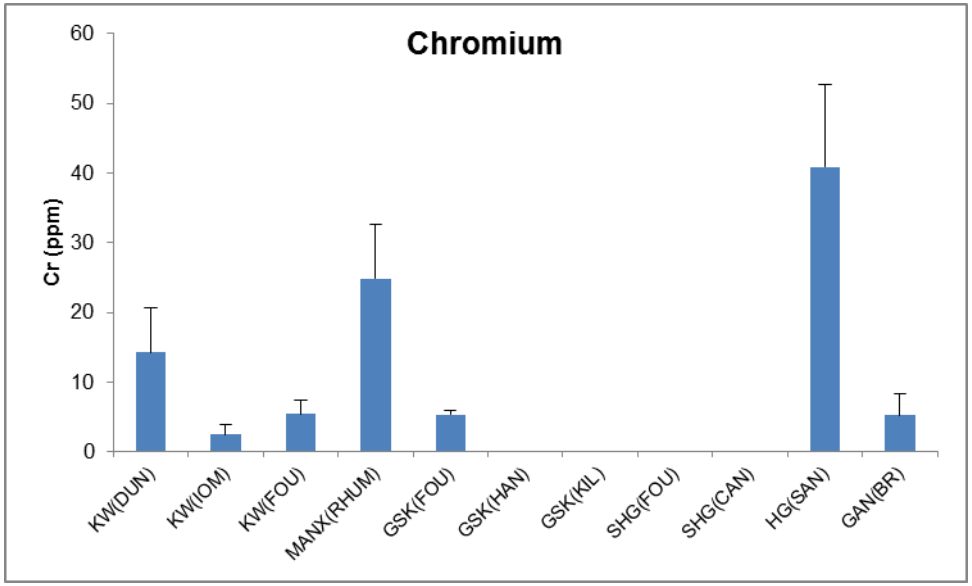

Iron
Fe (ppm)
3500
3000
2500
2000
1500
1000
500
0
KW(DUN)
KW(IOM)
KW(FOU)
MANX(RHUM)
GSK(FOU)
GSK(HAN)
GSK(KIL)
SHG(FOU)
SHG(CAN)
HG(SAN)
GAN(BR)

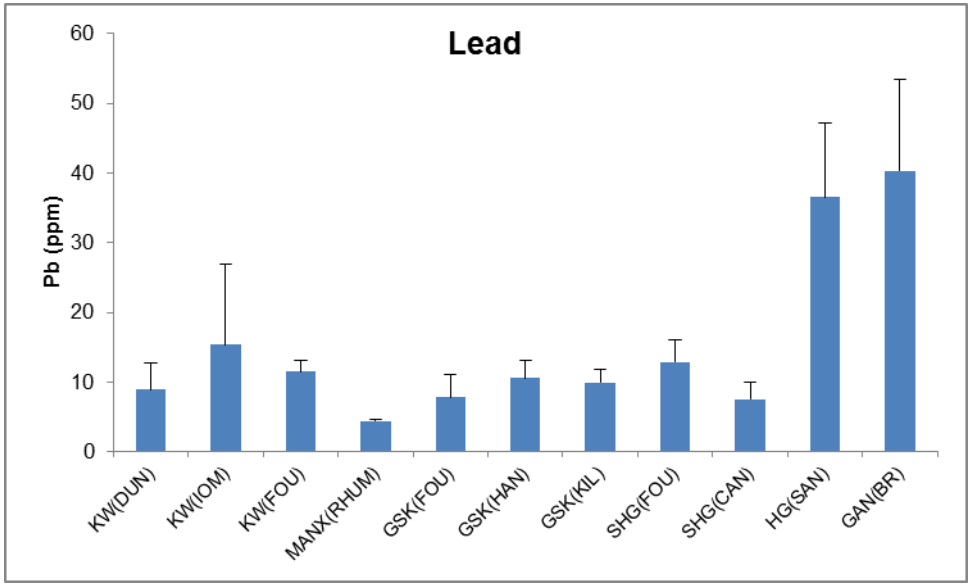

Copper
Cu (ppm)
50
45
40
35
30
25
20
15
10
5
0
KW(DUN)
KW(IOM)
KW(FOU)
MANX(RHUM)
GSK(FOU)
GSK(HAN)
GSK(KIL)
SHG(FOU)
SHG(CAN)
HG(SAN)
GAN(BR)


Zinc
Zn (ppm)
450
400
350
300
250
200
150
100
50
0
KW(DUN)
KW(IOM)
KW(FOU)
MANX(RHUM)
GSK(FOU)
GSK(HAN)
GSK(KIL)
SHG(FOU)
SHG(CAN)
HG(SAN)
GAN(BR)


Arsenic
As (ppm)
30
25
20
15
10
5
0
KW(DUN)
KW(IOM)
KW(FOU)
MANX(RHUM)
GSK(FOU)
GSK(HAN)
GSK(KIL)
SHG(FOU)
SHG(CAN)
HG(SAN)
GAN(BR)


Selenium
Se (ppm)
12
10
8
6
4
2
0
KW(DUN)
KW(IOM)
KW(FOU)
MANX(RHUM)
GSK(FOU)
GSK(HAN)
GSK(KIL)
SHG(FOU)
SHG(CAN)
HG(SAN)
GAN(BR)


Chromium
Cr (ppm)
60
50
40
30
20
10
0
KW(DUN)
KW(IOM)
KW(FOU)
MANX(RHUM)
GSK(FOU)
GSK(HAN)
GSK(KIL)
SHG(FOU)
SHG(CAN)
HG(SAN)
GAN(BR)


Lead
Pb (ppm)
60
50
40
30
20
10
0
KW(DUN)
KW(IOM)
KW(FOU)
MANX(RHUM)
GSK(FOU)
GSK(HAN)
GSK(KIL)
SHG(FOU)
SHG(CAN)
HG(SAN)
GAN(BR)

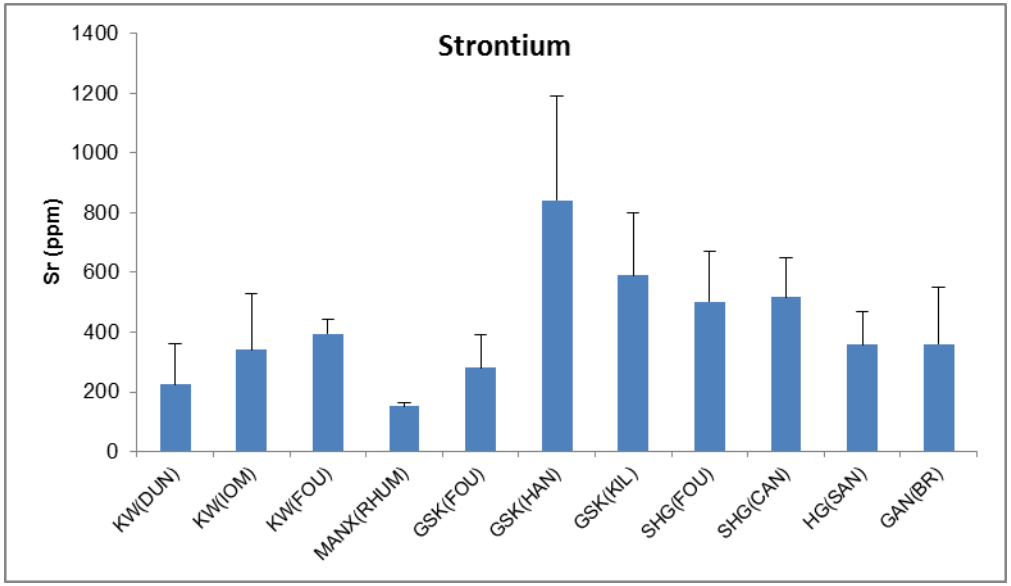


Figure 4.8. Individual value plots of trace elements in seabird guano for (a) Manganese, (b) Iron, (c) Copper, (d) Zinc, (e) Arsenic, (f) Selenium, (g) Chromium, (h) Lead and (i) Strontium. Bars are one standard deviation from the mean. Acronyms for each species are as follows: KW(DUN) – Kittiwake, Dunbar Harbour; KW(IOM) – Kittiwake, Isle of May; KW(FOU) – Kittiwake, Foula; MANX(RHUM) – Manx shearwater, Isle of Rhum; GSK(FOU) – Great skua, Foula; (GSK(HAN) – Great skua, Handa; GSK(KIL) – Great skua, St Kilda; HG(SAN) – Herring gull, Sanda; SHG(FOU) – Shag, Foula; SHG(CAN) – Shag, Canna; GAN(BR) – Gannet, Bass Rock.

significantly higher levels of Fe and Zn detected in guano than in 2008 and 2009, while levels of Sr were higher in 2007 than 2008.

There were some significant within year similarities in levels of trace elements in guano of kittiwakes in 2007 and 2009 (2008 was not considered as only a single month of sampling was undertaken). In 2007, samples from April showed significantly higher levels of Fe, Cu, Br, Pb, As and Se than the rest of the sampling months which were largely consistent, whereas Zn showed much more variability from May to July 2007. There was no significant in year differences for Mn, Cd and Sr. In 2009, there was significant differences between sampling months for Fe, Cu, As, Br, Sr and Pb, but no obvious difference for Mn, Zn, Cd and Se.

Dunbar Harbour

Guano collected from kittiwakes at Dunbar Harbour showed no significant differences in Mn, Fe and Pb across the sampling years (2007 to 2009) when the data was pooled together. In contrast, samples from 2009 showed significantly higher levels of Cu, Se, Br and Sr in guano than in 2007 and 2008, but lower levels of Zn. Levels of As were significantly lower in 2007 than other years.

There were significant within year differences in the guano of kittiwakes for several trace elements from 2007 to 2009. In 2007, there was no significant within year differences for As, Se, Sr and Cd. In comparison, June showed significantly lower levels of Mn, Fe, Cu, Br and Pb that the other months, whereas May showed significantly higher levels of Zn and Cr. In 2008, there was no significant within year differences in Cu, Zn, Se, Br, Sr and Cd, whereas As varied significantly across sampling months. Kittiwakes sampled in August showed significantly lower levels of Mn, Fe and Pb, while levels of Cr in June were higher than those detected in August. In 2009, kittiwakes sampled in June had significantly lower levels of most trace elements than other months, with the exception of Cr where there was no significant difference.

Chapter 5. Discussion

5.1 Analysis of seabird guano and uric acid as an indicator of seabird dietary patterns

Stable isotopic analysis has been increasingly applied to faecal studies in a range of mammals, including bears (Hatch et al. 2011), sheep (Martins et al. 2012), cattle (Sponheimer et al. 2003) and primates (Tsutaya et al. 2016) in order to understand dietary components and sources, with largely successful results. In comparison, there have only been very limited isotopic studies using guano and uric acid as dietary indicators, with a few examples from migratory song birds (Podlesak et al. 2005) and zebra finches (Bird et al. 2008). Given the scarcity of information currently available on the stable isotopic analysis of seabird guano and uric acid and, in particular its relationship with other biological materials, making comparison between studies can prove problematic (Bird et al. 2008). This difficulty can be further compounded when dietary components exhibit very little isotopic difference (Moreno et al. 2011) as a result of feeding on prey with similar isotopic signatures or in similar regions (Forero et al. 2004); or when diets are complex, consisting of many different components (Hatch et al. 2011). For example, this study found that kittiwakes generally exhibited similar nitrogen values as a result of their dependence on small pelagic shoaling fish (Wanless et al. 2005), while more generalist predators such as great skuas showed greater isotopic variability due to more varied diet (Figure 4,1 and Table 4.1, Results). Making inferences using stable isotopic analysis in these instances can therefore be difficult. In such cases, complementary conventional dietary studies, or alternative analytical analysis such as fatty acid analysis can prove valuable (Quinn, 2014; Tsutaya et al. 2016).

In the present study, only stable isotope analyses of guano and uric acid were undertaken, therefore the value of this method alone is limited if information on dietary components is required. Furthermore, interpreting isotopic data based solely on information in the literature is also fraught with difficulties (Moreno et al. 2011). However, the aim of the present study was not to undertake a full isotopic analysis of seabird diet, but rather to test the utility of guano as a potential method for determining the diet and dietary niches of seabirds. That said, dietary data for some

species and locations[7] were available for to the present study due to established conventional dietary studies at these sites, therefore where undertaken, these provided support to some conclusions made in subsequent areas of the discussion (section 5.2). Such data however, was not available for all colonies and species, and in all years, therefore the present study could not interpret the isotopic data to determine actual diet in the studied birds, but rather make assumptions and conclusions on the basis of information on seabird behaviour and diet available from other colonial studies (Phillips et al. 1997; Wanless et al. 2007; Guidford et al. 2008; Roscales et al. 2011, Newell et al. 2013a, b, c). For future work, consideration should be given to collecting conventional dietary data or utilising alternative analyses (e.g., fatty acid analysis), as well as extending stable isotope analysis to additional tissues, which have similar turnover times (e.g., blood) therefore increasing confidence in the dietary data.

The successful utility of guano or faeces as an effective indicator of an animal's diet, is understanding how $\delta^{13}C$ and $\delta^{15}N$ signatures reflect diet (Bird et al. 2008; Hatch et al. 2011; Martins et al. 2012). Similarities between the isotopic composition of faeces and diet isotopic composition has previously been observed for sheep, bats and birds (Bird et al. 2008; Martins et al. 2012), with these studies generally reporting diet-faecal discrimination in the range of <1‰ (Table 5.1), although some studies on mammalian herbivores suggest that this discrimination can be up to 3‰. For example, Steele & Daniel (1977) demonstrated that for cattle the $\delta^{15}N$ signatures of faeces was similar to that of the diet (within 2‰); a similar result to that reported by Bird et al (2008) who found that the $\delta^{15}N$ and $\delta^{13}C$ signatures of captive zebra finch guano was similar to its dietary intake prior to dietary switch.

Similarly, studies have also been undertaken on urine in relation to herbivores, with only one known study on uric acid. These studies demonstrated that $\delta^{13}C$ and $\delta^{15}N$ levels in urine were more depleted than faeces in relation to diet (Steele & Daniel, 1977; Sponheimer et al. 2003) although the difference were not considered

[7] Kittiwakes, Isle of May for all years (Newell, 2013) and Great skuas from Handa (Jones et al, 2008) in each of the sampling years.

Table 5.1. Stable isotope results from feeding trials comparing diet with stable isotopic composition of excreta and urine/uric acid. Errors representing 1 standard deviations from the mean of analysis are provided where available. Results are for excreta unless stated otherwise. (Gu=Guano, UA=Uric acid).

Species	**Diet composition**	**Carbon**		**Nitrogen**		**Reference**
		Diet (‰)	Tissue (‰)	Diet (‰)	Tissue (‰)	
Zebra finches, *T.guttata*		-16.0±0.3	Gu:-15.6±2.1 UA:-14.7±0.2	3.1±0.2	Gu: 4.1±0.3 UA: 4.3±0.1	Bird et al, 2008
		-25.4±0.2	Gu:-25.3±0.3 UA:-24.7±0.1	3.8±0.3	Gu: 4.1±0.1 UA: 4.4±0.1	
Chimpanzee, *Pan troglodytes*	Protein	-24.89±0.17	-27.04±0.29	4.14±0.17	5.96±0.28	Tsutaya et al, 2016
Sheep,	Corn silage	-13.67±0.10	-14.54±0.14			Martins et al, 2012
	M. sativa	-31.15±0.10	-31.60±0.37			
Llama, *Lama glama*	*M. sativa*			0.4	Fecal: 3.3±0.3 Urine: 0.1±0.4	Sponheimer et al, 2003
	C. dactylon			5.8	Fecal: 8.8±0.4 Urine: 3.7±0.2	
Alpaca (n=4)	*M. sativa* *C. dactylon*	-27.0±0.2 -13.3±0.3	-27.4±0.4 -14.7±0.2			Sponheimer et al, 2003
Cattle (n=4)	*M. sativa* *C. dactylon*		-28.0±0.2 -14.2±0.2			
Goat (n=4)	*M. sativa* *C. dactylon*		-27.7±0.1 -14.3±0.4			
Llama (n=4)	*M. sativa* *C. dactylon*		-27.4±0.5 -14.5±0.4			
Horse (n=4)	*M. sativa* *C. dactylon*		-27.4±0.4 -14.0±0.2			
Cattle	Hay	0.6	Urine: –0.7±0.1 Fecal: 2.6±0.1			Steele & Daniel, 1977
	Silage	0.6	Urine: -2.45±0.35 Fecal: 2.3±0.2			

statistically significant. This is in contrast to Bird et al (2008) who found that while the δ13C and $\delta^{15}N$ isotopic composition of uric acid in captive zebra finches (*Taeniopygia guttata*) were generally similar to the $\delta^{13}C$ and $\delta^{15}N$ of dietary intake; following a dietary switch, uric acid became enriched by 2‰ and 1.5-2.5‰ for $\delta^{13}C$ and $\delta^{15}N$ respectively, possibly as a result of slight differences in diet quality. Taking the results of the literature into account, it is possible that the isotopic signatures of guano and uric acid seen in the present study closely reflects the dietary intake of the birds studied, however without controlled dietary studies or knowledge of the isotopic composition of all prey species consumed, it is difficult to test this hypothesis.

5.1.1 Discrimination factors of guano and uric acid

Guano consists of a complex mixture of undigested material from the diet as well as previously digested material and intestinal slough (Hatch et al. 2011). A significant proportion of metabolic waste in birds is excreted as uric acid (often as high as 70-90%, Mizutani et el. 1985) which is derived from excess dietary nitrogen and protein catabolism (Bird et al. 2008), although urea and ammonia are also excreted in guano, generally making up <25% of excreted nitrogen (Bird et al. 2008). Similar to guano, uric acid preserves the isotopic composition of the food metabolized by the bird and can therefore be used to determine dietary habits.

The present study found that for most species the $\delta^{13}C$ signatures of uric acid were not significantly different from that of guano (<1‰) with a highly linear relationship, although there was some slight variation in some gull species which showed more variation between guano and uric acid with differences up to 2.5‰ (Figure 4.6, a-e), but this was still not considered statistically significant. These general findings are in agreement with Bird et al (2008) who found that prior to a dietary switch, captive zebra finches showed significantly similar $\delta^{13}C$ signatures for both uric acid and guano. However, following the dietary switch, Bird et al (2008) found that this discrimination slightly increased, although the authors could not be sure whether this was attributed to analytical uncertainties, slower leakage of ^{13}C into the uric acid pool or differences in food quality between diets. In the present study, where some of the gull species had slightly more enriched uric acid $\delta^{13}C$ signatures (compared to

guano) than in the other species, it is possible that this discrimination could be attributed to dietary differences suggested by Bird et al (2008) as gull species are known to be generalist predators with a high diversity of prey (Steenwag, 2010). Indeed, it was noted in the present species that gull species from Ailsa Craig tended to consume more terrestrial derived food sources which could have accounted for the guano-uric acid discrimination found in the present study.

In comparison, the present study found that the relationship between the $\delta^{15}N$ signatures for guano and uric acid shows greater variability, uric acid being slightly more depleted in $\delta^{15}N$ than guano for the same sample with an average difference of ±1.2‰ (Figure 4.7, a-e). However, it is important to note that for all seabird species, this difference was not significant. This result contrasts with that reported by Bird et al (2008) for zebra finches, who found that guano and uric acid $\delta^{15}N$ signatures were similar. It is possible that the differences seen in the present study could be attributed to the fact that free-living seabirds do not feed directly on plant material, therefore the non-uric acid components excreted (e.g., urea and ammonia) in seabird guano maybe higher in ^{15}N than the components excreted as a result of seed based diet. For example, Sponheimer et al (2003) found that urine was more depleted in $\delta^{15}N$ than faeces of llamas possibly as a result of differential utilisation of dietary nitrogen by animals on variable diets. It is therefore possible that the slight differences seen between guano and uric acid in the present study compared to Bird et al (2008) is a result of ammonia being ^{15}N-enriched compared to uric acid. Other factors, including dietary stress and energy expenditure can also influence the $\delta^{15}N$ isotopic signatures of animals including birds, resulting in excretory products being more depleted in ^{15}N (Kelly, 2000; Sponheimer et al. 2003;). Furthermore, some samples did show uric acid $\delta^{15}N$ values up to 2.5‰ lower than the guano from the same sample. While it may be possible that analytical uncertainties could have contributed to some of this difference, it is considered likely that this difference is mainly due to the free-living nature of seabirds which consume different prey components resulting in differential excretion of non-uric acid components, as discussed above. While this study cannot answer this specific question, it is clear that further work needs to be undertaken to understand the basis of diet-tissue fractionation and the role of excreted fluids and solutes in isotopic fractionation if we

are to fully realise the potential of stable isotope analysis in dietary studies.

5.1.2 Application of guano and uric acid to seabird studies (comparison with published stable isotope literature)

This is one of the first studies to investigate the use of stable isotopes from seabird guano to infer foraging behaviour of seabirds, most of the published literature focuses on tissues such as feathers, blood, plasma and muscle (Bearhop, 2003; Kakela et al. 2007; Votier et al. 2010; Quinn, 2014). The lack of comparators is compounded by the fact that stable isotopic signatures within an individual's tissues vary considerably due to discrimination factors, tissue heterogeneity, isotopic mixing models, and metabolic processes (Bond & Jones, 2009) which can have a considerable influence on the results obtained. Nevertheless, there is an increasing understanding and knowledge about the factors that influence variability between tissues as a result of both captive and wild experiments (Bird et al. 2008; Hatch et al. 2011; Martins et al. 2012; Tsutaya et al. 2016), which can help inform the current work, although much of this work has largely excluded seabirds.

The results reported in the present study on the stable isotopic concentrations of seabird guano and uric acid, are largely in the range of results reported for some tissues (e.g., particularly blood) in seabirds studies in the North Atlantic and North Sea, but depleted in relation to others (e.g., feathers). Table 5.2 collates the results from published literature on stable isotope analysis of tissues from a range of seabird species in this region. Stable isotopic avian studies generally report enrichment of $\delta^{13}C$ by -0.4 to 6.0‰ and $\delta^{15}N$ by 1 to 5.0‰ over the diet for a range of tissues including breath, blood, muscle, liver, collagen and feathers for a wide range of species (Hobson & Welch, 1992; Kelly, 2000; Podlesak et al. 2005; Herrera & Reyna, 2007); and in most cases guano is found to be depleted in both $\delta^{13}C$ and $\delta^{15}N$ in relation to most of these tissues. For example, using tissues sampled from free-living songbirds, Podlesak et al (2005) found that for all five species, guano had the most $\delta^{13}C$ depleted isotopic signatures compared to feathers, breath, RBC and plasma by up to 6‰; a pattern also seen in mammalian herbivores (Coates et al, 1991; Sponheimer et al. 2003; Martins et al. 2012). The results from the present study are largely consistent with these findings, as it found that the stable isotopic

composition of guano and uric acid from fulmars and great skuas were depleted in $\delta^{13}C$ and $\delta^{15}N$ by up to 6‰ in comparison to feathers (Thompson et al. 1995; Bearhop, 2003; Kakela et al. 2007; Quinn, 2014) from birds from the same colonies. Studies attribute the lower $\delta^{15}N$ levels in guano to the preferential excretion of ^{14}N due to fractionation associated with the production of nitrogenous waste (Kelly, 2000), although studies are increasingly questioning the role that ^{15}N-depleted excreta plays in determining tissue $\delta^{15}N$ (Sponheimer et al. 2003) in animal in a steady state, therefore supporting the need for further studies about diet-tissue fractionation.

Table 5.2. Stable isotope for seabird species from the UK. All are mean values, with standard deviation provided where available.

Species	Location	Tissue	Carbon (‰)	Nitrogen (‰)	Reference
F. glacialis	Eynhallow, Orkney	Feathers	-17.16±0.52[1] (n=24) -17.31±0.58[2] (n=20)	15.49±0.52[1] (n=24) 14.88±0.20[2] (n=20)	Quinn, 2014
	Little Saltee (UK)		-15.76±0.76[1] (n=17) -15.73±0.69[2] (n=4)	17.62±0.85[1] (n=17) 17.01±1.73[2] (n=4)	
	St Kilda		-16.68±0.31[1] (n=14) -17.03±0.33[2] (n=15)	15.37±0.64[1] (n=14) 14.46±0.35[2] (n=15)	
	Faroes		-17.98±1.36[1] (n=15) -17.67±0.35[2] (n=15)	15.27±0.38[1] (n=15) 14.92±0.43[2] (n=15)	
	N. Isles	Body feathers	-17.39±0.55 (n=30)	14.18±0.76 (n=30)	Thompson et al, 1995
	St Kilda		-17.33±0.42 (n=30)	13.65±0.75 (n=30)	
M. bassanus	Grassholm, Wales	RBC	-17.33±0.68 (n=32)	15.18±0.69 (n=32)	Votier et al, 2010
		Plasma	-18.79±.0.82 (n=32)	15.98±0.73 (n=32)	
	Bass Rock	Blood	-16.90 (n=11)	14.20	Kakela et al, 2007
C. skua	Foula	Blood	-16.6±0.4	12.1±0.6	Bearhop, 2000
		Feathers	-14.4±1.2	15.1±1.2	
		Body feathers	-14.3±1.4	14.1±0.9	
	St Kilda	Blood	-17.3±0.5	13.1±0.9	
		Feathers	-13.9±1.3	13.9±1.5	
		Body feathers	-13.9±1.2	14.2±1.4	
	Shetland (various)		-17.20 (n=76)	12.40	Kakela et al, 2007
P. aristotelis	Foula		-17.70 (n=14)	9.20	Kakela et al, 2007
U. aalgae	Foula		-17.35 (n=14)	12.20	Kakela et al, 2007
Auk *sps*	Foula	Muscle	-12.8±0.2	11.7±1.1	Bearhop, 2000
	St Kilda		-14.8±3.9	11.1±0.3	
P. puffinus	Rhum	Primary feather	-16.12±0.02	18.21±0.03	Roscales et al, 2011

[1] Males; [2] Females

In contrast, the present study found that the levels of $\delta^{13}C$ and $\delta^{15}N$ in the uric acid of Northern gannets *Morus bassanus* from Bass Rock were within the range of those reported by Kakela et al (2007) in the blood of gannets from the same location. Similarly, they are also consistent with levels reported by Votier et al, (2010) in blood plasma of gannets from Grassholm, Wales. Given that uric acid is a component of blood plasma (Bird et al. 2008), it is therefore not surprising that the levels of $\delta^{13}C$ and $\delta^{15}N$ in the uric acid of gannets is comparable to that of blood plasma. Furthermore, blood plasma has a turnover time similar to that of guano (in the order of a few days) (Hobson & Clark, 1992) therefore it would be expected that results are similar as they reflect the same period of dietary intake.

The outcome of the present study therefore supports the hypotheses that stable isotopic analysis of guano and uric acid can be used as an alternative tissue when investigating dietary behaviour of seabirds. However, this must be supported by a knowledge of discrimination factors, tissue heterogeneity, isotopic mixing models, and metabolic processes (Bond & Jones, 2009) which can have a considerable influence on the results obtained.

5.2 Using C and N isotopes in understand the foraging ecology and dietary patterns of Scottish seabirds during the breeding season.

Using stable isotopic analysis of seabird guano and uric acid, the present study showed notable inter- and intra-specific differences in $\delta^{13}C$ and $\delta^{15}N$ signatures across breeding locations (Table 4.1 and Figure 4.1, Results; Figure 5.1). These differences are primarily influenced by the feeding ecology of each species, although aspects of spatial variation play a key part in some colonies due to the differentiated availability of fishery resources (Lewis et al, 2001; Votier et al. 2010). Although stable isotope analysis is frequently used to infer trophic ecology and seasonal movement of seabirds (Karnovsky et al. 2008; Votier et al. 2010), the present study highlights the difficulties that are inherent in studies that do not take account of the isotopic signatures of potential prey and oceanic isotopic baseline signatures, or the interpretation of isotopic data based solely on information available in the literature (Barnes et al. 2009; Moreno et al. 2011). However, the aim of this study is not to undertake a full analysis of seabird diet, but to test the utility of seabird guano as a

potential source of monitoring dietary patterns in seabirds against the backdrop of knowledge of foraging ecology and isotopic results reported in the literature. Table 5.1 collates the results from published literature on stable isotope analysis of tissues from a range of seabird species in the North Atlantic Ocean and North Sea.

Stable carbon and isotope values of seabirds in the present study were largely in accordance with expectation from the literature. Species with the lowest $\delta^{13}C$ values, indicating pelagic foraging were Manx shearwaters (*Puffinus puffinus*) and Northern fulmars (*Fulmarus glacialis*), while species known to feed predominantly inshore, such as European shags (*Phalacrocorax aristotelis*) exhibited a more enriched mean $\delta^{13}C$ value. Similar inter-specific patterns were also seen in nitrogen, with Northern gannets (*Morus bassanus*) having the highest $\delta^{15}N$ signatures indicative of foraging at higher trophic levels.

5.2.1 Interspecific differences in the foraging ecology and diet of Scottish seabirds.

The present study demonstrated that $\delta^{13}C$ and $\delta^{15}N$ signatures of seabird guano varied among species and regions (Figure 5.1). In the case of $\delta^{13}C$ isotopes, Northern fulmars and Manx shearwaters showed the strongest pelagic signatures of all species, as well as the greatest diversity in $\delta^{15}N$ isotopic signatures, indicating a varied diet and considerable foraging flexibility within the population. In the case of fulmars, this conclusion is consistent with previous research which shows that these birds have a wide-ranging prey base, comprising of whitefish, sandeels, crustaceans and fishery discards (Garthe et al. 2004; Roscales et al. 2011). The research also showed large foraging ranges during the incubation (2500 km, Edwards et al. 2013) and chick rearing periods (580 km, Weimerskirch et al. 2001) which is likely to account for the variation in stable isotopic signatures reported in this study. Similarly, Manx shearwaters show a wide spectrum of isotopic values, which is consistent with their foraging ecology. Utilising telemetry techniques, Guilford et al (2008) reported a mean foraging trip duration of 71.8 h (range: 2 to 12 days) in Manx shearwaters from Skomer Island, Wales during the breeding season, with trips consisting of localised foraging in Cardigan Bay, to more extended trips in the wider Irish Sea. This may explain the apparent wide variation in $\delta^{13}C$ isotopes seen in this study, although

without similar tracking studies it is not possible to determine whether individuals from Rhum exhibit similar patterns of both inshore and offshore foraging behaviour. However, Roscales et al (2011) did report that birds from Rhum were feeding in the Northern Irish Sea in response to increasingly favourable feeding conditions. The wider variation seen in $\delta^{15}N$ isotopes (6.5 to 13.1‰) however, cannot be so easily explained. While Manx shearwaters are known to be pelagic foragers with a diet comprised of fish, cephalopods and crustaceans (Gray & Hamer, 2001; Guidford et al. 2008), the variation seen in the present study is much greater than that reported in previous studies (Roscales et al, 2011). It is possible that this is linked to wide variation in feeding behaviour amongst individuals, with birds feeding in inshore waters having more enriched $\delta^{15}N$ due to longer food chains compared to the open ocean (Forero et al. 2004). However, in the absence of data on the isotopic signatures of all potential prey items in the Manx shearwaters foraging range, and conventional dietary studies at the study site, it is difficult to make full inferences about dietary patterns (Moreno et al. 2011). It has previously been suggested that differences in $\delta^{13}C$ isotopic values similar to those seen in fulmars and Manx shearwaters can be driven by latitudinal variation in baseline $\delta^{13}C$ signatures (Quinn, 2014) due to large distances over which these species range. However, published literature of the Atlantic Ocean isoscape reveals similar baseline $\delta^{13}C$ contours throughout the region (Graham, 2010); therefore it seems likely that the variation observed in the present study is a result of dietary behaviour as opposed to any other environmental factor.

In contrast, the present study showed that Black-legged kittiwakes (*Rissa tridactyla*), European shags, Great skuas (*Catharacta skua*), Arctic skuas (*Stercorarius parasiticus*), Northern gannets and Razorbills (*Alca torda*) generally had more enriched $\delta^{13}C$ values indicative of inshore/benthic foraging (Moreno et al. 2011), although the variability in isotopic signatures appeared to be dependent on the individual breeding colony. For example, kittiwakes, razorbills and shags from Foula (Shetland Isles) had low variation in both $\delta^{13}C$ and $\delta^{15}N$ values which is consistent with the strong dependence of these specialist species on a narrow range of small lipid-rich pelagic shoaling species, such as lesser sandeels and sprat (Wanless et al. 2005), although it is likely that the slightly more enriched $\delta^{13}C$ signatures observed in

shags is due to their tendency to feed in more inshore waters, often only up to 10km from the colony (Soanes et al. 2014). In contrast, the study reported greater variability in $\delta^{13}C$ signatures for great skuas, Arctic skuas and herring gulls (*Larus argentatus*). In the case of great skuas and herring gulls that are known generalists which feed on a wide diversity of prey, such carbon isotopic variation is to be expected (Kakela et al. 2007; Votier et al. 2003; Steenwag, 2010). However, the wide variation found in Arctic skuas from Foula and Handa cannot be so easily explained. Arctic skuas obtain the majority of their food through kleptoparasitism on other seabirds; at Handa this is primarily on razorbills and guillemots (Jones, 2002). Consequently, we would expect Arctic skuas to have similar $\delta^{13}C$ signatures as razorbills; however the greater diversity seen in the present study indicates that the sampled individuals were not only taking food from seabirds, but possibly opportunistically taking advantage of more benthic-inshore food sources due to the enriched $\delta^{13}C$ signatures found in some of the samples.

Wide variation in the $\delta^{13}C$ signatures in the guano of common (*Larus canus*) and herring gulls from Sanda and Burnt Island were also observed in the present study (Figure 5.1), although corresponding $\delta^{15}N$ signatures showed no similar consistency. While such variability can be easily explained due to the generalist nature of herring gulls in particular (JNCC, 2012), it was interesting to note that similar patterns were not seen in gulls from Ailsa Craig and Foula where carbon isotopic variance was generally low and typical of more benthic/inshore foraging. Such disparity between these species is likely to be linked to spatial differences and foraging behaviour of herring gulls at these individual colonies. Indeed, Steenwag (2010) reported that herring gulls in the Bay of Fundy, switched from a diet dominated by fish, to a varied diet incorporating fish, marine invertebrates and muskrats within a 5-week period, highlighting the generalist nature of this species.

In comparison, nitrogen isotopes showed much less variability across species and breeding colony (ranging from 6.5 to 13.5‰), relating to 1 to 2 trophic level differences (Hobson, 1999). In the present study, most sampled species and breeding colonies (76%) were grouped in a narrow isotopic range (about 4‰ of mean $\delta^{15}N$ signatures). Trophic similarity does not necessarily mean dietary

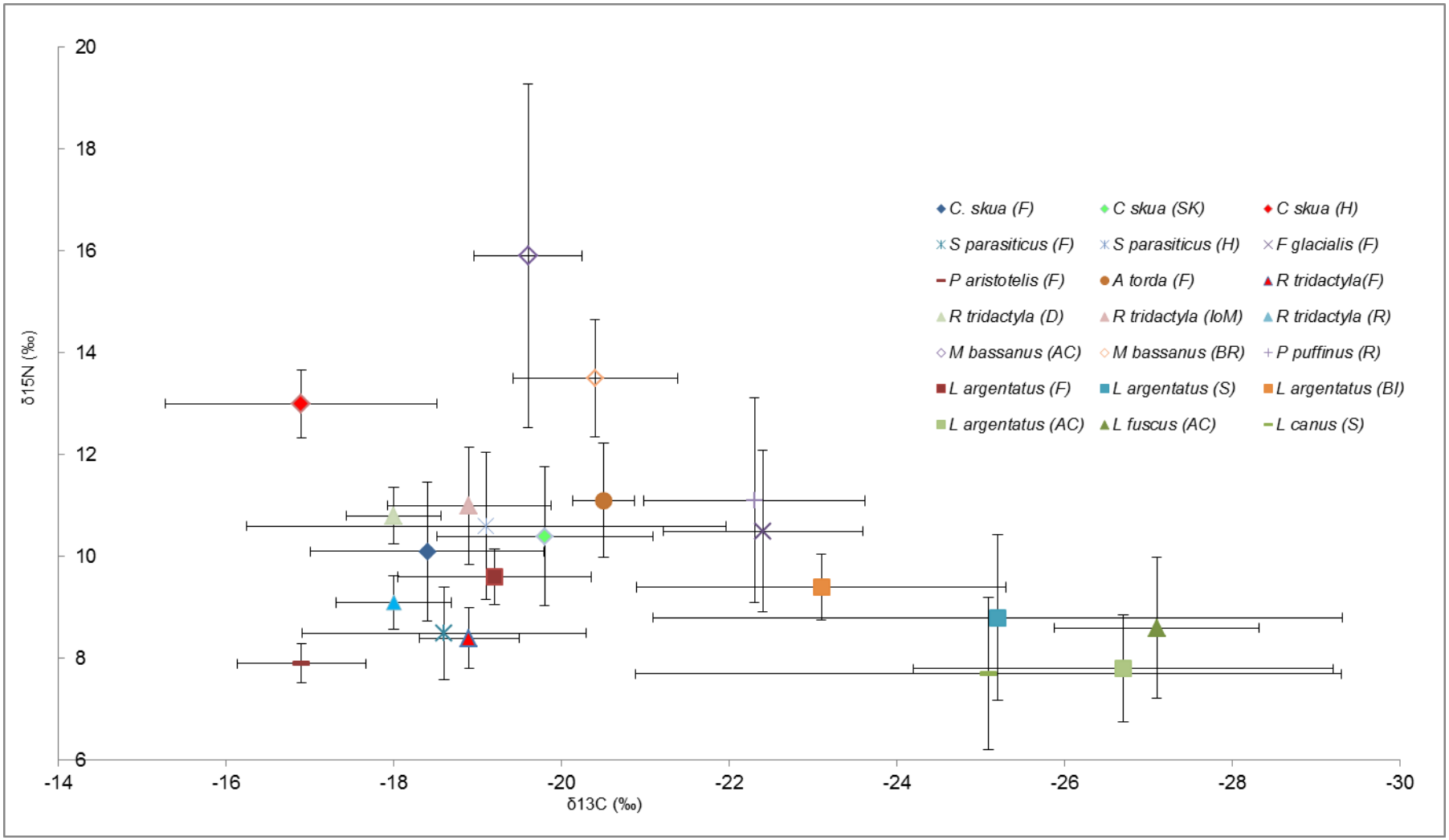


Figure 5.1. Mean±SD stable carbon and nitrogen isotope concentrations in guano of seabirds from Scottish colonies. Locational codes are as follows: F-Foula, SK-St Kilda, H-Handa, D-Dunbar, IoM-Isle of May, R-Rhum, S-Sanda, AC-Ailsa Craig, BI-Burnt Island. Bars present one standard deviations.

similarity because targeted prey species can occur at the same trophic level. Nonetheless, Forero et al (2004) suggested few dietary alternatives as the cause of overlap in $\delta^{15}N$ signatures of seabirds in Patagonia. This is also likely to be the case in the North Atlantic and North sea region, where the majority of seabirds in the present study are known to forage on similar fishery resources (JNCC, 2012; Lewis et al. 2001; Wanless et al. 2007), although the relevant proportions and size classes targeted are known to vary between species (Lewis et al, 2001; Wanless et al, 2005). However, it is important to note that increased competition for fishery resources at larger colonies or during periods of declining marine food availability, may result in diversification in the type of prey consumed by seabirds (Phillips et al. 1997) or increase in foraging range (Votier et al. 2010) which can influence consumer $\delta^{15}N$ isotopic signatures.

Similar to their $\delta^{13}C$ signatures, the present study shows that great skuas and herring gulls (Sanda and Burnt Island) also exhibited wide variation in their $\delta^{15}N$ isotopic values (in the order of 5 to 6‰). This is consistent with previous studies which show that these birds display a wide spectrum of diets across a population, as well as large individual specialisation in diet (Votier et al. 2004 & 2007; Jones et al. 2008; Steenwag, 2010). Phillips et al (1997) reported that great skuas on Foula had a diet dominated by seabirds, fish and fishery discards; a pattern also seen in colonies at St Kilda and Handa, although the relative proportions were different between colonies and largely driven by fluctuations in food availability, energy requirements and predatory specialisation of individual birds (Bearhop et al. 2001; Lewis et al. 2001; Votier et al. 2007).

Unsurprisingly, this study found the highest $\delta^{15}N$ signatures in Northern gannets from Bass Rock and Ailsa Craig, which is in agreement with the large body of literature on this species (Hamer et al. 2009; Garthe et al. 2011). Gannets are large seabirds that benefit from flexibility in diets and foraging behaviour which enable them to successfully buffer against variations in prey availability (Garthe et al. 2011). While gannets are known to feed on a wide diversity of prey, Hamer et al (2009) noted that the main components of their diet are typically sandeels (*Ammodytes marinus*), mackerel (*Scomber scomber*), herring (*Clupea harengus*) and sprat (*Sprattus*

sprattus), with group 0 sandeels generally making up the largest proportion. Such findings however, are in contrast to this study which found that the sampled gannets were eating at a higher trophic level than that reported for sandeels in this area (Sara, 2009). Without conventional dietary results for the year of study, it is difficult to provide an answer for this apparent discrepancy, but Quinn (2014) found that sandeels were entirely absent from the diet of gannets sampled on Bass Rock in 2010, and numbers in 2011 were very small (13%) in comparison to large mackerel (mean length of 24.2cm) which made up the majority of the diet in both 2010 and 2011. Since the data from gannets in the present study were collected in 2008, it may be that this year was also dominated by mackerel, but without dietary information it is not possible to derive further conclusions. Whatever the cause, one reason for the success of gannets over recent years may be their ability to switch to alternative prey items which are too large to be taken by most other seabirds (Frederiksen et al. 2007), and it is possible that it is this selectively that has resulted in the high $\delta^{15}N$ signatures seen in this study.

5.2.2 Intra-specific differences in dietary pattern at Scottish breeding colonies revealed by C and N isotopes

As well as differences between species, the isotopic data collected through the present study revealed strong evidence of within- and between-colony differences in diet for some species. Regional differences in diet composition have been shown to exist in a number of species, particularly during the breeding season (Frederiksen et al. 2007; Votier et al. 2010), and are largely driven by feeding behaviour and variations the availability of fishery resources within foraging range of the colony (Phillips et al. 1999; Mallory et al. 2010). In the present study the stable isotopic signatures of four species were investigated across their breeding colonies to reveal patterns of intra-specific differences between spatially distinct colonies.

Great skua, *Catharacta skua*

Great skuas are dietary specialists which feed on a wide variety of prey including birds, fish, shellfish, as well as practicing kleptoparasitism from other birds and scavenging at fishing vessels (Bearhop et al. 2001; Votier et al. 2004). However, the foraging strategy employed by individual birds is variable both within and between

colonies (Phillips et al. 1997; Jones et al. 2008) and inter-annually (Miles, 2010), and often influenced by the availability of food within foraging range of the colony. For example, Votier et al (2004) noted that reductions in the availability of fishery discards and of small shoaling pelagic fish such as sandeels in the North Sea resulted in some individuals adopting more specialised foraging strategies, most notably feeding almost exclusively on other seabirds. Similar patterns were also observed in the St Kilda archipelago where reductions in the availability of inshore spawning Capelin resulted in increased predation on Leaches storm-petrels, *Oceanodroma leucorhoa* (Miles, 2010).

In the present study we found some significant intra-specific differences in stable isotope signatures between colonies, with birds sampled on Handa exhibiting enrichment in both $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures. Studies of great skua diet from all three colonies revealed that while fish, fishery discards and seabirds are the main dietary components, the proportions of each can often vary between colonies and between years (Votier et al. 2004 & 2007; Kakela, 2007). In the case of birds from Handa whose diet is evenly split between fish and birds (Smith & Jones, 2007), it is possible that the large consumption of Norway Pout (*Trisopterus esmarkii*) and Whiting (*Merlangius merlangus*) in 2007 could account for the differences observed at Handa due to these demersal species being enriched in both $\delta^{13}C$ and $\delta^{15}N$ (Kakela et al. 2007). Indeed, previous research by Votier et al (2010 & 2013) reported enriched $\delta^{15}N$ signatures in fulmars and gannets that consumed a greater proportion of fishery discards, possibly due to the larger size and age class of fish. Similarities in $\delta^{13}C$ signatures between Foula and Handa could also be attributed to consumption of these demersal species as feeding on discards are as important component of skua diet at Foula (Lewis et al. 2001).

Interestingly, the apparent similarities seen in the $\delta^{13}C$ and $\delta^{15}N$ signatures of birds sampled at St Kilda and Foula are not consistent with published literature which reports distinct dietary differences between the colonies (Phillips et al. 1999; Votier et al. 2004 & 2007). In the absence of conventional dietary data for these colonies during the sampling period, or a full knowledge of the isotopic signatures of potential prey and baseline isotopic signatures, it is difficult to make assumptions about

dietary patterns, particularly when comparing similar isotopic signatures (Moreno et al. 2011). However, it is clear from the results, that while skuas at both colonies are taking a wide range of prey, the isotopic similarities seen between colonies may not necessarily imply diet consistency since some prey species are known to have similar isotopic signatures (Forero et al. 2004), but rather that they are foraging on prey at similar trophic positions. Furthermore, it should be noted that observations undertaken at Foula (*Furness, pers comms)* suggest that seabird predation by skuas was much higher than normal in 2006, which could potentially contribute to some of the similarities seen between colonies.

Black-legged kittiwake, *Rissa tridactyla*

Overlap in $\delta^{13}C$ and $\delta^{15}N$ values between colonies suggests that individuals from different colonies may be feeding on prey items with similar isotopic signatures or in similar regions (Forero et al. 2004). This appears to be the case for kittiwakes from the Isle of May and Dunbar Harbour which exhibited similar $\delta^{15}N$ values, most likely due to their high dependence on small pelagic shoaling fish such as sandeels, predominantly at Wee Bankie (Wanless et al. 2005, Frederiksen et al. 2007). Similarities in $\delta^{13}C$ isotopes also appear to support this assumption, although the minor disparity with birds from Dunbar showing slightly more enriched δ13C signatures is most likely a reflection of disparity between sampling months[8] as opposed to differences in spatial foraging patterns.

The lower $\delta^{15}N$ levels (up to 2‰) observed in birds from Foula and Rhum, and the lack of any apparent overlap with east coast colonies may be a reflection of feeding on smaller sized fish, with narrow variation indicative of a diet based on a single prey type, most likely sandeels (Furness, 2002; Frederiksen et al. 2004). Since sampling at Foula and Rhum was undertaken in a single month (July) as opposed to most of the breeding season (as was the case for the east coast colonies), it could be suggested that this disparity was due to over-representation of 0-group sandeels in samples from Foula and Rhum, while the east coast colonies were also influenced by larger 1+ class sandeels. However, similar differences were seen when only July

[8] Samples were collected in April 2007 on the Isle of May, with these samples showing a more depleted $\delta^{13}C$ than the rest of the sampling months.

was considered, so it is likely that larger prey items, coupled with the consumption of additional prey items, is likely to account for the higher $\delta^{15}N$ signatures on the east coast. Indeed, kittiwake dietary data gathered on the Isle of May during 2007, found much higher levels of clupeids and pipefish in regurgitations than previously reported at this colony (Newell et al. 2013a) supporting this hypothesis. Interestingly $\delta^{13}C$ signatures were relatively consistent across all breeding colonies, suggesting that birds from all colonies are foraging in isotopically similar conditions; a view supported by Roscales et al (2011) who found that most populations of Procellariiformes from the same species grouped according to their isotopic values regardless of their breeding colony when studying the geographic structure of birds in the northeast Atlantic.

Northern gannet, *Morus bassanus*

The lack of any overlap in $\delta^{13}C$ signatures of gannets from Ailsa Craig and Bass Rock suggests that individuals are feeding on prey items with different isotopic signatures as a result of different foraging habits (Forero et al. 2004). Gannets are flexible foragers, able to exploit a wide range of species and sizes of prey, but lipid-rich fish such as mackerel and sandeels and demersal whitefish from commercial fisheries appear to be the most consistent prey at UK colonies (Votier et al. 2010); this prey type consistency is reflected in the $\delta^{15}N$ signatures reported in the present study which were similar across both breeding colonies. Consequently, it is likely that the disparity in $\delta^{13}C$ between colonies is a response to gannets feeding in favourable foraging conditions. Indeed, Davies (2012) found that gannets from Ailsa Craig undertook shorter foraging trips in 2009 in response to more favourable feeding conditions close to the colony in the northern Irish Sea, that were also reflected in large increases in populations of razorbills and guillemots at nearby colonies (Allen et al. 2011) which are likely to be related to increasing prey availability. It is therefore possible that the shorter foraging trips, resulting in more localised feeding, could be a factor in the more enriched $\delta^{13}C$ signatures seen in birds from Ailsa Craig in this study.

In comparison, gannets from Bass Rock exhibit lower $\delta^{13}C$ signatures (Table 4.1, Results) which is likely to be a result of feeding in more pelagic waters. This

assumption is in agreement with previous literature which found that gannets from Bass Rock largely focused their foraging at a range of bathymetric features including Dogger Bank, Halibut Bank, Buchan Deep and Farne Deep, possibly in response to enhanced primary productivity (Hamer et al. 2001; Hamer et al. 2007). Given the disparate geographical locations of these colonies, variation in the baseline $\delta^{13}C$ signatures could have been a factor in the distinct differences seen between the colonies. However, published literature of the Celtic and North Sea (Barnes et al. 2009) reported relatively consistent baseline $\delta^{13}C$ contours between the regions, thus supporting the conclusion that the variation observed is a result of foraging behaviour.

Herring gull, *Larus argentatus*

Herring gulls are generalist predators, feeding on large range of prey, including fish, fishery discards, marine invertebrates, other birds and terrestrial species (Nogales et al. 1995; JNCC, 2012). As generalist species, herring gulls exhibit a great deal of foraging flexibility, which may change on a seasonal or annual basis, as well as varying between breeding colonies (Steenwag, 2010). Indeed, in the present study we found that herring gulls showed variability in isotopic signatures, particularly $\delta^{13}C$, within and between colonies reflecting this foraging flexibility, although there was evidence of some prey consistency when consuming marine species. This was the case for all colonies, with the exception of Ailsa Craig, where birds foraging in the marine environment exhibited similar isotopic signatures to other seabirds within the colony. For example, the present study found that herring gulls on Foula had similar $\delta^{15}N$ and $\delta^{13}C$ isotopic ranges to most sampled seabirds with the exception of fulmars and shags. Kleptoparasitism by herring gulls at seabird colonies has been previously reported in the literature (Steenwag, 2010) therefore it may be that the consistency of results between species is due to this specialised foraging strategy.

In the case of gulls from Sanda, there was a positive correlation between $\delta^{13}C$ and $\delta^{15}N$ isotopic values, with individuals feeding on terrestrial-influenced prey sources exhibiting lower $\delta^{15}N$ signatures than those feeding on marine prey. This could be a result of inshore waters having more enriched $\delta^{15}N$ due to longer food chains because of benthic-pelagic coupling (Forero et al. 2004). Exploitation of terrestrial-

influenced resources was also seen in gulls from Ailsa Craig, with all individuals sampled feeding solely in this environment. While there is little recent information on herring diet at Ailsa Craig, Nogales et al (1995) found marked differences between parental diet and that of chicks with adult diet comprising 90% of terrestrial food, while chicks fed predominantly on fish in the early weeks, before decreasing in quantity in favour of meat. Nogales et al (1995) hypothesised that such dietary choice in adults was a result of reduced competition for terrestrial resources and the abundance of anthropogenic food resources in foraging distance of the colony. Similar changes in chick diet were also reported by Steenwag (2010), therefore it may be that the isotopic signatures seen in gulls from Ailsa Craig in the present study are a result of similar foraging behaviour.

5.2.3 Inter- and intra-annual variation in the foraging behaviour of the black-legged kittiwake, *Rissa tridactyla* at two east coast colonies

Inter-annual variation in the diet of seabirds has been widely reported in scientific literature, largely in response to fluctuations in food availability and changing energy requirements or reproductive constraints during the breeding season (Phillips et al. 1997; Moreno et al. 2011). Indeed, Phillips et al (1997) reported annual differences in the contribution of sandeels to the diet of great skuas, presumably as a reflection of changes in availability in waters surrounding the breeding colonies. Similarly, lesser sandeels, *Ammodytes marinus* are known to be important in the diet of kittiwakes during the breeding season (Coulson & Thomson, 1985; Oro & Furness, 2002; Frederiksen et al. 2004; Daunt et al. 2008), with their inter-annual presence indicating that birds from the Isle of May and Dunbar Harbour are exploiting a relatively predictable prey resource. Indeed, birds from these colonies feed predominantly on sandeels obtained from Wee Bankie, with the occurrence of 0+ group fish coinciding with the kittiwakes breeding season (Frederiksen et al. 2007). However, the relative abundance, size and body condition of sandeels can fluctuate significantly between years (Wanless et al. 2005; Newell et al. 2013a, b) with these fluctuations largely reflected in the contribution of sandeels to the diet of kittiwakes, as well as their isotopic signatures (Newell et al, 2013a, b, c).

The present study observed considerable isotopic overlap between sampling years

(Figure 5.2), suggesting that individuals from these colonies were consuming isotopically similar prey, taken largely from similar regions (Forero et al, 2004). However, there was evidence of some slight fluctuations in isotopic signatures with birds sampled in 2007 exhibiting enriched $\delta^{13}C$ and $\delta^{15}N$ signatures (Table 4.1, Results), although in the case of the Isle of May, the $\delta^{15}N$ enrichment was observed in 2009 (Figure 5.2). It is possible that the fluctuations seen in the isotopic signatures of birds in 2007 were a reflection of dietary changes in kittiwakes at these colonies. Indeed, dietary data from kittiwakes on the Isle of May[9] during the sampling period reported some significant changes in the diet of kittiwakes in 2007, with the occurrence of sandeels in regurgitations at their lowest level since records began (Table 5.2). Newell et al (2013a) reported that sandeels comprised of 47.9% of total biomass in 2007, which was nearly half of that reported in 2009, while the presence of clupeids and pipefish in regurgitations increased markedly. Similarly, kittiwakes at Dunbar Harbour exhibited increased consumption of pipefish and gadoids (mainly rockling) as the 2007 breeding season progressed (*pers observ*). It is therefore possible that the presence of these species in the diet of kittiwakes, could have contributed to the isotopic enrichment seen in 2007, although factors such as changes in foraging area (Quillfeldt et al. 2005), body condition (Hobson et al. 1993), and nutritional stress (Hobson et al. 1993) which are also known to influence the isotopic signatures of seabirds should not be ignored. Indeed, it has been widely reported in the literature that animals subject to nutritional stress can exhibit enriched ^{15}N similar to those causing trophic level nitrogen fractionation (Bond & Jones, 2009) as a result of enhanced protein catabolism (Hobson et al. 1993; Cherel et al. 2005a). For example, Voigt et al. (2003) observed unpredictable enrichment in ^{15}N in nectar-feeding bats which they attributed to the excreted 'lighter' nitrogen not being replaced by dietary protein, resulting in the animal becomes progressively more ^{15}N enriched within increased stress. Furthermore, since uric acid levels in avian blood have been correlated with dietary stress (Bird et al. 2008), it is possible that the ^{15}N enrichment seen in the guano in 2007 could be a result of this process. The 2007 breeding season was the worst on record since the 1990s with productivity well

[9] The Isle of May is one of a number of UK colonies studied annually as part of the Seabird Monitoring Programme (SMP). The aim of these studies is to monitor the breeding success, adult survival and diet of 26 seabird species. The Centre for Hydrology and Ecology (CEH) undertakes the monitoring on the Isle of May on behalf of the Joint Nature Conservation Committee (JNCC).

below the long-term average (Newell et al. 2013a), therefore it is possible that this effect, compounded by fasting during incubation and searching for food, could potentially have been a contributing factor to the results found in the present study.

Interestingly, the present study found that birds from Dunbar Harbour exhibited a steady decrease in $\delta^{13}C$ and $\delta^{15}N$ signatures between 2007 and 2009 (Table 4.1-Results), which coincided with an increase in the number and biomass of sandeels in the diet of kittiwakes from the Isle of May (Table 5.3). While we do not have dietary data from Dunbar Harbour for the years sampled in this study, birds from this colony feed at Wee Bankie along with birds from the Isle of May (*pers comms, Centre for Hydrology and Ecology*) so it is possible that the changes in isotopic signatures are a reflection of this dietary change.

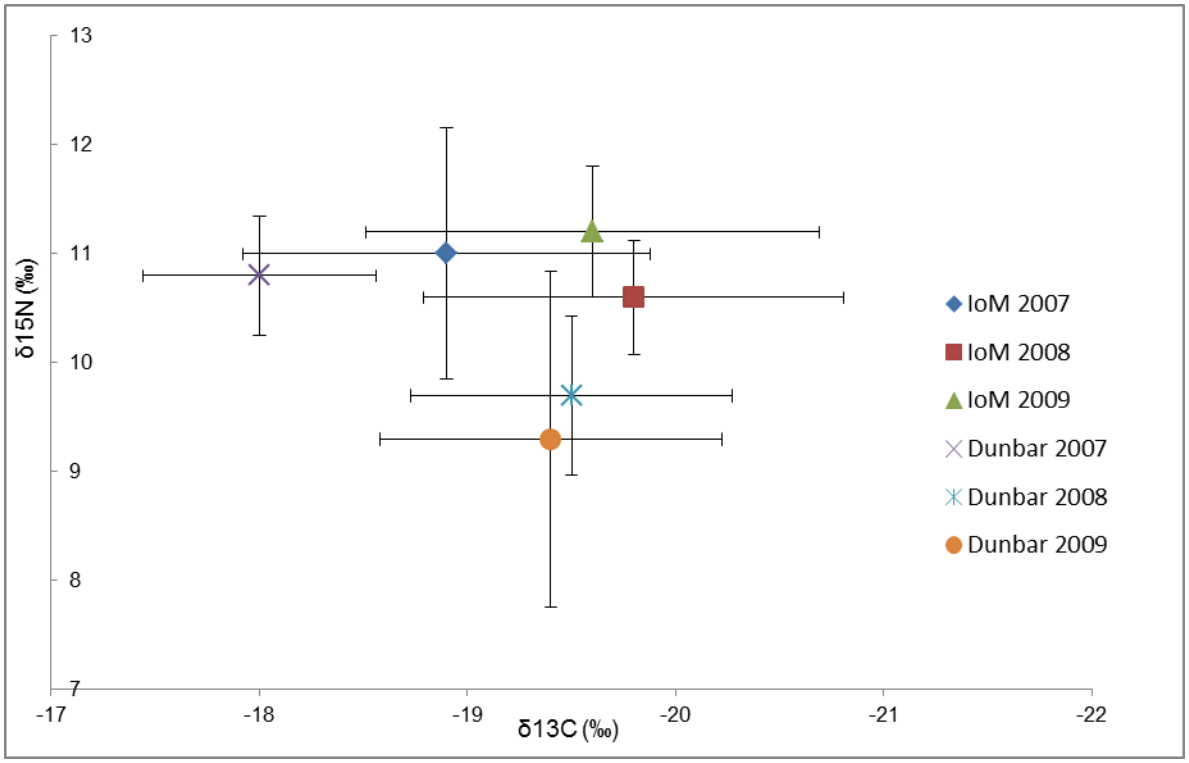


Figure 5.2. Mean carbon ($\delta^{13}C$) and nitrogen isotope ($\delta^{15}N$) results for guano concentrates for kittiwakes at the Isle of May (IoM) and Dunbar Harbour (2007 to 2009). The bars represent one standard deviation.

Intra-annual variability in diet during breeding is well reported for some seabird species due to the individual energy requirements of both adults and chicks (Steenwag, 2010; Wanless et al. 2005). However, the current study found limited

aspects of intra-annual isotopic variability within the colonies, suggesting that birds were largely feeding on prey of similar isotopic signatures and in the same foraging regions throughout the breeding season; this is largely in general agreement with published literature on kittiwake foraging behaviour at these colonies (Lewis et al. 2001; Wanless et al. 2007). The present study did find evidence of nitrogen enrichment later in the breeding season (June) which could be a result of an increasing proportion of isotopically enriched prey in the birds diet. Indeed, evidence from diet studies at breeding colonies highlights the increasing proportion of $\delta^{15}N$ enriched fish in the diet of individual kittiwakes as the breeding season progresses.

Table 5.3. Regurgitations in the diet of kittiwakes on the Isle of May in 2007, 2008 and 2009.

	2007	2008	2009
% of total regurgitations			
Sandeels	70.5%	76.1	96.3
Clupeids	39.3%	56.5	35.0
Pipefish	39.3%	39.1	0
Others species	variable [1]	variable [1]	Minimal
% of total biomass			
Sandeels	48.0	58.6	87.4
Clupeids	26.1	20.5	6.3
Pipefish	NA[2]	NA[2]	0

[1] In 2007 and 2008 there were unprecedented numbers of rockling in the diet for the first time. However, as they were small pelagic immature fish, it was not possible to identify them to species level. [2] Biomass of pipefish cannot be quantified as they are bony with little representation in biomass.

5.2.4 Utilising C and N isotopes to infer seabird dietary and trophic relationships across geographical regions

Stable isotopic analysis has been increasingly employed in recent years to infer diet and trophic relationships and migratory patterns of seabirds (Furness et al. 2006; Forero & Hobson, 2003). Subsequently, this has resulted in a greater knowledge and understanding about some of the limitations and caveats of the methodology, as well as the assumptions that are made in relation to discrimination factors, tissue heterogeneity, isotopic mixing models, and metabolic processes (Bond & Jones, 2009). While these factors are generally well known and subsequently applied in isotopic studies, some areas relating to the use of stable isotopes in the marine

environment are not so well considered. These are particularly important when considering isotopic variations across different geographical scales, which were done as part of the present study.

In recent years, a number of studies have discussed the potential implications of oceanic baseline isotopic signatures on C and N signatures, particularly when making inferences about a species trophic level and foraging locations. These become increasingly relevant when making isotopic comparisons over large temporal and spatial scales (Forero et al. 2004; Barnes et al. 2009). Since the present study sampled different populations and colonies between 2006 and 2009, it is possible that the results could have been influenced by inter-annual variability in baseline isotopic signatures (Forero et al. 2004). Indeed, Moreno et al (2011) reported that isotopic baseline signatures can cause variation in $\delta^{13}C$ and $\delta^{15}N$ values by up to 2‰ which could be misconstrued as a trophic level response in terms of $\delta^{15}N$. In the case of this study, wide scale isoscapes produced by Barnes et al (2009) found that there was minimal change in the carbon isotopic baseline in the Celtic, North Sea and English Channel beyond that known for the gradient between benthic/pelagic and inshore /offshore environments; so it is unlikely that changes in baseline isotopic signatures had a bearing on isotopic relationships given the small variation in latitude between the colonies. Indeed, Roscales et al (2011) found no evidence of a latitudinal gradient in carbon, and reported that inter- and intra-species variation was likely to be greater than any such effect. That said, it is likely that a latitudinal gradient is present in oceans, therefore there is a need to better understand their isotopic signatures (or isoscapes) when considering potentially large geographical and temporal changes.

Seabird species are known to forage preferentially at areas of high productivity, notably frontal zones, upwellings and shelf edges (Weimerskirch, 2007), therefore it is likely that seabird $\delta^{15}N$ signatures are not only influenced by diet, but also natural oceanic processes. Indeed, from isotopic studies along the west coast of Spain, Moreno et al (2011) frequently found simultaneous influences on the seabirds' $\delta^{15}N$ signatures by both baseline isotopic signatures and foraging in areas of enhanced productivity. Similar findings were also reported by Hamer et al (2001 & 2007) who

found that gannets from Bass Rock largely focused their foraging at a range of bathymetric features including Dogger Bank, Halibut Bank, Buchan Deep and Farne Deep, most likely in response to enhanced primary productivity in these areas. As a result, when considering all the caveats and assumptions associated with this methodology, these factors must also be considered, taking account of the growing literature in these areas.

5.3 Trace elements in Scottish seabird populations

The present study, which investigated concentrations of nine essential (Mn, Fe, Zn, Cu, As, Cd, Sr) and non-essential trace elements (Pb, Cd) in the guano of six seabird species (Black-legged kittiwakes, *Rissa tridactyla*; Great skua, *Stercorarius skua;* European shag, *Phalacrocorax aristotelis;* Atlantic gannet, *Morus bassanus;* Herring gull, *Larus argentatus*), from Scottish colonies, demonstrated wide variation in trace element concentrations for some species. Such a response would be expected as guano reflects a bird's dietary intake (De Moreno et al. 1997), and since different species have varying life history strategies, behaviour and physiology, ecology, dietary habits and habitat use; trace element concentrations will vary accordingly (Burger & Gochfeld, 2004, Kaur Kler et al. 2014). Furthermore, the processes by which metals accumulate in the body, and are subsequently excreted, are species-dependent and also related to the mechanisms of detoxification and metabolism (Jakimska et al. 2011).

In the marine environment the main route of exposure to trace elements is through ingestion of food and water (Burger 1993, Jakimska et al. 2011). Once ingested, the element can be stored or accumulated within the body, or excreted, with birds known to purge their body of metals through excreta or guano (Dauwe et al. 2000). Most studies investigating trace element levels in seabirds have typically focused on eggs, feathers, blood and other internal tissues such as liver and kidney (Furness & Camphuysen, 1997; Thompson et al. 1998; Becker et al. 2002). However, more recently less invasive methods such as excreta (guano) have been studied, although these are still rare with only a handful of studies reporting trace element concentrations in seabird guano, primarily from penguins and other seabirds in the Southern Ocean (Yin et al. 2008; Metcheva et al. 2011; Celis et al. 2012 & 2014;

Wing et al. 2014).

Given the limited information currently available for trace element concentrations in seabird guano and its relationship with other biological materials, making comparison between studies can prove problematic (Ancora et al. 2002). Consequently, a small number of studies comparing guano with other materials such as feathers, eggs and stomach contents (Ancora et al, 2002. Metcheva et al. 2011) have been undertaken. Investigating trace element levels in feathers, eggs and excreta of Gentoo penguins, Metcheva et al (2011) reported significantly higher levels of all trace elements, with the exception of Pb, in excreta compared to other materials; in some cases these levels were up to 40 fold higher. Similar results were also reported by Yin et al (2008) who found the highest concentrations of Pb, Zn and Cu in the guano of a diverse range of seabirds from the Arctic, Antarctic and South China Sea; in some cases levels in guano were up to 60-fold higher. Ancora et al (2002) suggested that this was a result of poor element absorption by the gastrointestinal tract, and also to the ability of the kidney and liver to eliminate excess metal levels which ensures that guano is the material with the highest concentrations of trace elements. Furthermore, Friberg et al (1986) noted that only 5% of ingested Cd was absorbed into the body with the rest being excreted through faeces; a pattern also noted by Kaur Kler et al (2014) who reported that levels of Cd, Mn and As in birds were regulated primarily by excretion in faeces. These findings are therefore consistent with the view that guano has higher levels of trace elements to other material as a result of preferential excretion (Duawe et al. 2000); a finding that could explain the elevated levels of some trace elements seen in this study.

While guano levels are a direct reflection of a species dietary uptake and can provide information on the levels of metals at a particular point in time (Dwaue et al. 2000), caution must be applied when interpreting these results since physiological and biochemical processes governing elemental absorption, can affect concentrations excreted (Ancora et al. 2002). However, it is generally perceived that dietary differences as a result of differential foraging behavior is likely to affect trace element levels more than metabolic processes (De Moreno et al. 1997; Yin et al. 2008). Nonetheless, this study has allowed the investigation and comparison of the

relationship between the feeding habits of several seabird species and their trace element levels, with the aim of testing the feasibility of using seabird guano as bio-monitors of trace element concentrations.

Finally, the lack of published data on trace element levels in guano of the seabird species considered as part of this study makes it difficult to discuss the results. For that reason it was necessary to compare the results of the present study with those data contained from other seabird studies from other locations although where possible, relationships with seabirds in this geographical range were discussed.

5.3.1 Trace elements concentrations

The results reported in the present study for trace element concentrations in guano, with the exception of lead (Pb), are largely within the ranges reported in seabirds studies in Antarctica, Arctic and Southern China Sea (Metcheva et al. 2011; Celis et al. 2012; Wing et al. 2014), despite different geographical locations. Liu et al (2013) reported that such patterns of similarities between global regions are to be expected as general element composition of the marine environment and of seabird prey are relatively stable. Table 5.4 collates all known information on trace element levels in excreta/guano taken from the literature.

Metal concentrations in guano reflect the unabsorbed metal content in food items and excretion of absorbed metals (Metcheva et al. 2011), with diet being suggested as a major factor of element variation, most likely as a result differential foraging behaviour and dietary habits (Furness & Camphuysen, 1997; Burger & Gochfeld, 2004, Kaur Kler et al. 2014). In the present study, concentrations of Mn, Fe, Cu, Zn, As and Cd showed significant similarities between species, with the exception of herring gulls and gannets which showed elevated levels across most elements. Such a response would be expected as these species are piscivorous predators, feeding at roughly the same trophic level, although herring gulls and gannets are known to have more diverse dietary requirements (Hamer et al. 2007; Davies, 2012). Indeed, Savinov et al (2003) reported that species with more heterogeneous diets tended to have much higher heavy metal concentrations than species with homogeneous diets, although this may not be the case in all elements.

Table 5.4. Levels of trace elements in the guano of a selection of marine birds (mean values represented in ug g^{-1}).

Species	Location	Pb	Cu	Fe	Zn	Mn	Cd	As	Reference (paper cited)
Glaucous gull (*Larus hyperboreus*)	High Arctic	30[1]	6.25[1]		76[1]				Headley (1996)
Black-legged kittiwake (*Rissa tridactyl*)	High Arctic	21.6[1]	51.2[1]		176[1]				Headley (1996)
Yellow-legged gull (*Larus cachinnans Pallas*)	Cies Islands, North-west Spain	29.9[1]	60.1[1]		305.1[1]		5.8[1]		Otero Perez (1998)
Red-footed booby (*S. sula*)	Dongdao Island, South China Sea	1.6[1]	21.1[1]		419.4[1]		6.34[1]		Liu et al (2006)
Brown skua (*Catharacta skua*)	Auckland Island, New Zealand			6550	415	54	<2	2	Wing et al (2014)
Black-backed gull (*Larus dominicanus*)	Auckland Island, New Zealand			750	60	30	74	13	Wing et al (2014)
Auckland Island shag (*Phalacrocorax colensoi*)	Auckland Island, New Zealand			2250	230	22	17	6.5	Wing et al (2014)
Gentoo penguin (*Pygoscelis papua*)	Livingston Island, Antarctic Peninsula	<0.40	104	185	145	12.3	1.03	5.12	Metcheva et al (2011)
Gentoo penguin (*Pygoscelis papua*)									Mendes et al (2008)
Gentoo penguin (*Pygoscelis papua*)	Antarctica: Site 1-O'Higgins Site 2-Videla	 0.40 0.75					 0.8 1.4	 0.2 0.5	Celis et al 2012
Humboldt penguin (*Spheniscus humboldti*)	Northern Chile 1. Pan de Azucar 2. Chanaral Island 3. Cachagua Island	 1.80 1.59 12.79	 147.79 69.62 199.67		 487.11 222.55 0.83		 47.70 21.24 42.47	 1.84 0.36 7.86	Celis et al (2014)
Red-footed booby (*Sula sula*)	Dongdao Island, South China Sea	0.1	22.0		470				Yin et al, 2008
Giant petrels (*Macronectes giganteus*)	Great Wall Station, West Antarctica	3.8	98.5		167				Yin et al, 2008

Concentrations are represented as either $mgkg^{-1}$ dry mass[1]

Consequently, this study presents some significant inter-specific similarities and differences between results which can largely be explained by dietary habits and foraging location.

For essential elements Mn, Zn, Fe, Se, Cr and Cu, concentrations are closely regulated in the body of seabirds (Kim et al. 1998). Concentrations of these elements in the guano of kittiwakes, great skuas and shags in this study were in the range of those reported for other seabird species (Table 5.4). For example, they were consistent with those reported by Wing et al (2014) who found Mn, Fe, Cd, Cu and Zn levels in the guano of coastal foragers of similar magnitude. Similarly, Savinov et al (2003) found corresponding concentrations of Cu, Zn and Se in the hepatic fluids of inshore feeders (e.g., black-legged kittiwakes, herring gulls, razorbills and guillemots) in the Barents Sea which they attributed to dietary intake. In another study, Celis et al (2014) found similar levels of Fe, Mn, Zn in the excreta of Gentoo penguins, although their Cu levels were more elevated, most likely a result of prey preference. Interestingly, in our study we found that concentrations of Zn and Fe varied between species, with great skuas and shags showing higher concentrations of Zn than Fe, while herring gulls, gannets, and kittiwakes had elevated Fe concentrations in their guano. In the tissue and organs of vertebrates Fe levels are usually higher than Zn (Jerez et al. 2011); a pattern that is also seen in excreta (Metcheva et al, 2011; Kaur Kler et al. 2014). The transfer of essential elements, like zinc, in the food web is modulated by homeostatic mechanisms in organisms which strive to keep levels physiologically adequate (Dauwe et al. 2000) which may explain the lower Zn levels seen in some of the birds in this study.

The results presented in this study for As range from less than 1ppm (Manx shearwater) to 12.41ppm (kittiwake, Dunbar), although in most cases they were less than 3.5ppm. Little information has been reported about As levels in guano of seabirds, but when comparing our As levels with those from other species, they are consistent with the concentrations reported by Wing et al (2014) for the Brown skua (2ppm), black-backed gull (13ppm) and Auckland shag (6.5ppm), and by Mecheva et al (2011) for Humboldt penguins (5.13ppm). Interestingly, great skuas from all locations reported some of the lowest levels of As; a result consistent with Wing et al

(2014) who found that lowest As levels in the guano of predatory foragers. Wing et al (2014) also found the highest levels in coastal feeders which they attributed to feeding extensively in the intertidal environment on mussels and crustacean. This suggestion could partially explain the elevated levels seen in herring gulls and kittiwakes from the Isle of May; in the case of the latter, we know that kittiwakes feed on crustacean early in the breeding season, so the elevated As levels seen in April and May could account for this dietary behaviour.

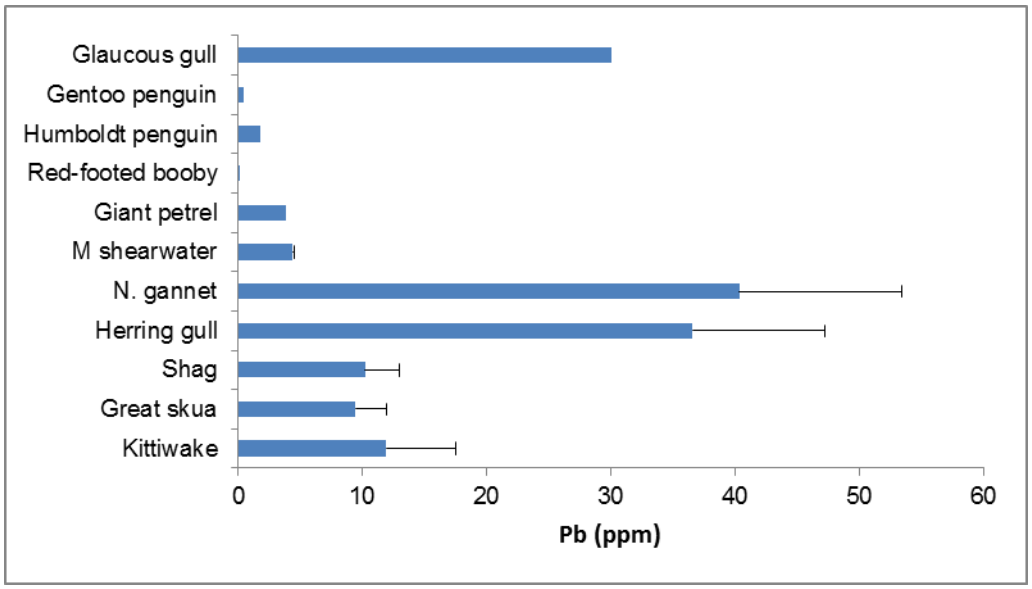


Figure 5.3. Mean concentrations (ppm) of Lead (Pb) in guano from seabird species in this study (shearwater, gannet, herring gull, shag, great skua and kittiwake) and from collated literature (first five values). Bars represent one standard deviation from the mean.

While the literature clearly shows that high metal levels are consistently reported in seabird species (Nisbet, 1994), the elevated Pb levels reported in the present study cannot be easily explained. There is very little data available on Pb levels in marine birds (Borga et al. 2006; Jakimska et al. 2011), and that which is available for guano, reports consistently low Pb levels. This can be explained by the strong affinity of Pb to epidermal tissues such as hair, bone, nails and feathers which results in an accumulation of Pb in these tissues (Jerez et al, 2011). For example, Metcheva et al (2011) reported Pb levels in feathers 4-fold higher compared to that in guano of Gentoo penguins; while Pb levels in the feathers of Great tits (*Parus major*) were

only 1.5 fold higher than excreta levels (Costa et al. 2013). In contrast, Ancora et al (2002) found the opposite to be the case with Pb levels in the guano of *Pygoscelis adeliae* being 20-fold higher than in feathers, which they attributed to more preferential excretion of dietary Pb via guano. Similarly, Duawe et al (2000) reported that when Great tits (*Parus major*) were exposed to high levels of Pb, they preferentially excreted the excess through their guano. Despite this conflict regarding the preferential excretion route for dietary Pb, it is clear that the levels found in this study appear to be higher than 10ug g^{-1} (dry weight) as described in guano from other bird species (Figure 5.3), thus suggesting that the majority of species in this study have a high Pb dietary burden, with both herring gulls (36ppm) and gannets (40ppm) showing highly elevated levels which are clearly in the region of physiological effects (Celis et al. 2014). Consequently, in the absence of available literature about the absorption, accumulation and excretion of Pb in marine birds (Jakimska et al. 2011), it is clear from the results presented in this study that further information is required to understand whether these results are a true reflection of Pb burden in these seabirds.

In addition to the similarities reported between species, the present study also found some significant differences; most notably the high trace element concentrations for gannets and herring gulls. These differences are likely to be attributed to their highly diverse diet, and in the case of gannets, their higher trophic status; although other contributing factors cannot be ruled out. In the case of herring gulls *Larus argentatus* the study found that they had some of the highest levels of Fe, Mn, Zn, Cr and Pb of the species studied. Herring gulls are generalist species, feeding in diverse habitats and on a large range of prey, including fish, small birds, invertebrates, bivalves, eggs and terrestrial species (JNCC, 2012) and therefore prone to accumulating high levels of particular trace elements. Such findings are consistent with Otero Perez (1996) who reported that elevated levels of Pb and Zn in the guano of Yellow–legged gulls (*Larus michahellis*) was attributed to the birds feeding on municipal rubbish tips, while Savinov et al (2003) found high levels of Zn in the in soft tissues of herring gulls from the Barents Sea indicative of their diverse foraging habits. In the case of Mn, the levels reported in the present study were 3 fold higher than Wing et al (2014) reported in the guano of black-backed gulls, and over 6 fold higher than Metcheva et

al (2011) reported in Gentoo Penguins. It is possible that these elevated levels are due to the herring gull regulating their dietary intake of Mn through excretion, although it is important to note that very little known is known about Mn absorption, accumulation and excretion and therefore further studies are required (Kaur Kler, 2014). Another potential factor for the elevated trace element levels in herring gulls could be the location of Sanda at the head of the Clyde Estuary, which has localised contaminated sediment as a result of historical discharges, as well as diffuse inputs which increase during high rainfall (Scottish Government, 2012) which could result in localised accumulations in their prey species.

In the case of Northern Gannet, *Morus bassanus*, the present study reported high levels of guano Fe, Cu, Se, Zn and Pb. The gannet is the largest pelagic seabird species in the North Atlantic, feeding on lipid-rich pelagic fish such as mackerel, herring and older age-class sandeels (Hamer et al. 2007; Davies, 2012) as well as discards from fishing vessels (Camphuysen et al. 1995). In the context of very limited data on trace elements in *Morus bassanus*, Mendes et al (2008) reported similar concentrations to the present study for Mn, Cu and Se in gannets in Portugal, but much lower levels of Pb and Zn; such findings they stated were within previously reported ranges for other seabird worldwide. The Se and Cu concentrations reported here are also consistent with the findings from a range of seabird species in the Barents Sea (Savinov et al. 2003), with the higher levels of Cu possibly a result of increased consumption of copper-containing food (Savinov et al. 2003) or species-specific variation in their requirements for Cu. A potential reason for the increased trace element concentrations reported in gannets from the present study could be that they occupy a higher trophic level and, therefore accumulate some metals more intensively (Kaur Kler et al. 2014). Trophic level relationships have been reported for a range of species and for a number of contaminants (Burger, 2002), and such relationships could therefore be attributed to the bioaccumulation of different metals in food items, although it is important to note that studies have also found no such relationships (Borga et al. 2006; Anderson et al. 2010). Another potential explanation for the high levels is that gannets from Bass Rock are known to feed on the Dogger Bank fishing grounds in the North Sea (Hamer et al, 2007), therefore these elevated levels could be associated with feeding in this area where there is a high volume of

vessel movement. Indeed, Metcheva et al (2011) and Celis et al (2014) suggested that the high elemental levels exhibited by penguins in Antarctica could be attributed to anthropogenic activities in the surrounding areas, most notably vessel movements.

In addition to the elevated levels found in herring gulls and gannets, the present study also reported some notable low concentrations. In particular, the study found that great skuas from Foula exhibited the lowest levels across all elements with the exception of As and Se, where Manx shearwaters had the lowest levels. Interestingly the other species sampled at Foula at the same point in time (e.g., kittiwakes, shags) did not exhibit this same pattern, with the exception of Cu and Zn for both and Fe for shags only. Patterns of geographical differences in trace element concentrations have been reported in the literature, and could be applied to the similarities seen in the three Foula species for Cu and Zn. For example, Celis et al (2012, 2014) found significant differences among the different locations for several trace elements which they attributed to variation in anthropogenic activities and pollutant runoff. As a relatively pristine environment (Phillips et al. 1997), it is fully expected that species from Foula would have reduced element levels compared to other areas (e.g., North Sea, Firth of Clyde), which can be seen in the Cu, Zn and Fe levels. However, it does not explain the consistent low levels seen for great skuas. Great skuas from Foula are known to feed on a mixture of sandeels, gadids and other seabirds (Voiter et al. 2003; Kakela et al. 2007), therefore dietary habitats could not be the only explanation for this observed pattern, other factors including the birds physiological and biochemical processes could be potential contributors.

5.3.2 Intra-specific differences

When considering intra-specific differences between species, Mendes et al (2008) reported that variation in trace element concentrations was likely to be a result of dietary variation. In the case of this study, levels of Mn, Zn, As, Pb, Cr and Cd in great skua guano showed no significant differences between the three study locations (St Kilda, Handa and Foula), but guano concentrations of Cu, Se, Fe and Sr were significantly lower at Foula compared to the other colonies (Figure 5.4). A possible reason for this pattern could be that Foula samples were collected a year

earlier in 2006, representing temporal as well as spatial variation which has been reported in other studies (Burger et al. 2015). However, it is more likely this intra-specific variation is attributed to differences in the diet of skuas between the colonies (Stewart et al. 1997; Jones et et al. 2008); an assumption supported by dietary studies which found that skuas from Handa and St Kilda exhibited high predation on other seabirds (63% and 53% respectively) and larger fish (Jones et al. 2012) which would account for increased elemental burden at these colonies.

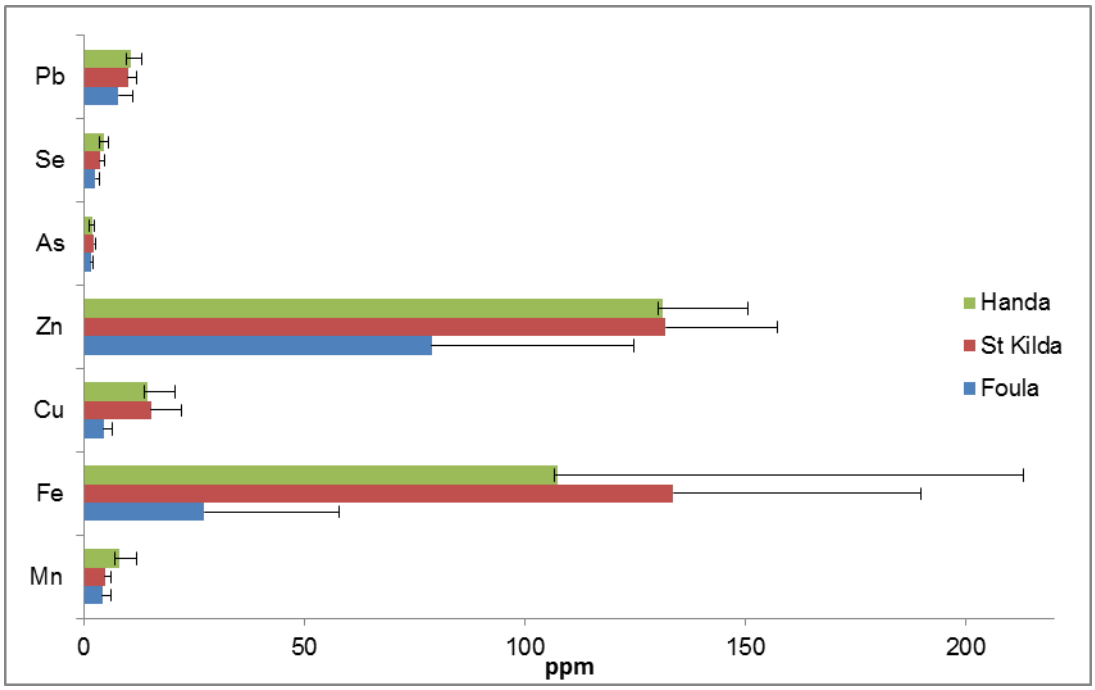


Figure 5.4. Mean concentrations (ppm) of seven trace elements in the guano of great skuas from three Scottish breeding colonies. Bars represent one standard deviation from the mean.

Black-legged kittiwakes also exhibited anomalous patterns in metal concentrations between locations (Figures 5.5 a, b). Birds from Dunbar harbour had the highest concentrations of Mn, Fe, Cu, Zn and Cr, while As and Pb were highest in birds from the Isle of May; birds from Foula had significantly lower levels of Cu and Zn than the other colonies, although As concentrations were largely similar across all three sites. Similar to the results seen in great skuas at Foula, some of this variation could be attributed to temporal factors due to different sampling years and months, but it is likely that dietary variability plays a major role with birds from the Isle of May and

Dunbar harbour probably feeding on larger and older fish and on a slightly more varied diet (Wanless et al. 2007; Newell et al. 2013a). Kittiwakes in all three colonies primarily feed on 1+ group sandeels in April and May, 0+ group sandeels in June and July, before changing back to 1+ group fish in August onwards, but birds from Dunbar and the Isle of May are known to feed on demersal species (e.g., herring and sprat) from July onwards (*pers comms*) which could contribute to the higher trace element levels seen in these colonies. Geographical factors such as increased vessel traffic from shipping and fishing in the kittiwakes feeding grounds in the North Sea cannot be ruled out as a potential contributory factor to these higher levels. An interesting additional observation was the highly elevated Fe levels observed in birds from Dunbar Harbour which are 6 to 8 folder higher than kittiwakes from the other colonies (Figure 5.5 and Table 5.4). Little is known about the dietary habits of birds from Dunbar, but it is thought that they feed at Wee Bankie along with birds from the Isle of May, although fish discards from vessels in the harbour are known to form an important component of their diet (Harris and Wanless, 1997; Coulson & Macdonald, 1962). Since mechanical errors during analysis were ruled out as a possible cause of these levels, it is probable that these individuals are subject to a higher Fe burden which could be related to feeding in the harbour where oil and vessel traffic are prevalent.

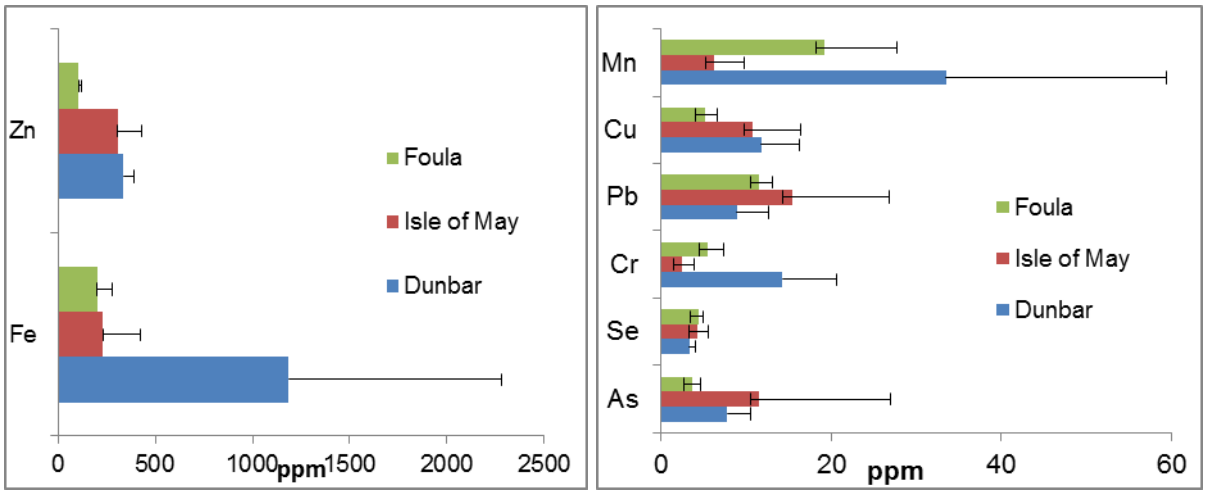


Figure 5.5 (a, b). Mean concentrations (ppm) of eight trace elements in the guano of kittiwakes from three Scottish breeding colonies. Error bars represent standard deviation.

5.3.3 Temporal differences in trace elements

Focused studies on inter-annual variation in the trace elements concentrations of seabird guano have not been widely reported. The present study therefore provides the opportunity to investigate differences in trace element concentrations in kittiwake guano at two breeding colonies.

Inter-annual differences in the diversity and availability of fish in the diet of seabirds have been extensively reported (Jones et al. 2008; Wanless et al. 2007; Burger et al, 2015) and is to be expected that this variability would contribute to yearly differences in trace element concentrations. Indeed, Seixas et al (2005) found significant inter-annual differences in the concentrations of Cu, Se, As and Cd in the arms of *Octopus vulgaris* which they suggested could have been a result of the movement of sediment which could have affected prey availability. Similarly, Lucia et al (2016) found increasing levels of Cd and Pb in the feathers of Ivory gulls (*Pagophila eburnea*) between 2012 and 2014, although they attributed this to changes in contaminant pathways and new anthropogenic pressures.

Table 5.5. Concentrations (ppm) of nine trace elements in the guano of kittiwakes sampled on the Isle of May and at Dunbar harbour between 2007 and 2009. Data shown are means ± one standard deviation.

	Isle of May			Dunbar harbour		
	2007	2008	2009	2007	2008	2009
Mn	6.1±3.6	4.1±.1.0	4.3±0.9	33.5±26.0	29.4±21.0	24.6±12.9
Fe	231.1±194.6	52.3±37.0	83.1±70.0	1187.0±1093.2	1184.9±1124.8	1133.7±715.2
Cu	10.8±5.6	3.7±1.4	7.1±3.1	11.8±4.5	10.8±4.1	23.7±11.0
Zn	306.0±126.9	202.7±28.5	171.0±32.1	335.8±56.3	367.4±127.0	262.0±68.4
As	11.5±15.5	3.7±1.6	5.5±4.2	7.7±2.7	14.4±18.9	27.9±19.5
Se	4.2±1.4	3.6±0.6	4.3±0.7	3.3±0.7	3.6±0.6	4.7±1.3
Sr	341.9±185.6	174.7±68.1	281.2±165.5	225.9±133.7	216.0±61.8	621.4±360.3
Cd						5.7±1.3
Cr	2.5±1.4	9.9±10.5	2.6±1.4	14.3±6.4	7.3±0.8	9.6±3.8
Pb	15.4±11.5	8.7±2.5	12.0±3.3	8.9±3.8	5.3±4.3	8.8±6.3

In the present study we found that while most trace elements were not significantly different between years for both colonies, we found that birds sampled on the Isle of May in 2007 had higher concentrations of Zn and Fe than subsequent years, while Dunbar Harbour birds had elevated levels of Cu and Se in 2009 (Table 5.5). It is likely that the elevated levels of Fe seen in Isle of May birds in 2007, are linked to the

variance seen within 2007 (and also 2009) where birds sampled early in the breeding season had significantly elevated levels of Fe, Cu, Pb and As compared to later in the season. Kittiwakes from breeding colonies in the Firth of Forth are known to feed on crustaceans in April (CEH, *pers comms*) which could have contributed to these elevated levels, a view supported by Savinov et al (2003) in their study on kittiwakes.

A similar seasonal pattern was also seen in birds from Dunbar Harbour which exhibited lower levels of Mn, Fe, Cu and Pb in June in each year of the study (2007 to 2009), as opposed to the other months, although interestingly the resulted showed that levels of these elements did appear to increase again towards July and August. It is likely that the lower concentrations in June are linked to kittiwakes dietary preference for 0+ group sandeels (Harris & Wanless, 1997; Newell et al. 2013a, b, c) which may have lower concentrations due to their smaller size and age (Frakas et al. 2003), while feeding on older and larger fish at other times of the breeding season could partially explain the higher levels in these months. Despite this, it is clear than there are inter-annual differences in trace element concentrations, therefore emphasising the importance of including more than one year of samples to understand trace element bioaccumulation. Environmental factors such as temperature and precipitation might also effect trace element concentrations contributing to the inter-annual variation and should be considered as potential additional contributory factors (Markowski et al. 2014; Berglund et al. 2015).

Chapter 6. Conclusions

This study investigated the potential of seabird guano for monitoring environmental changes in (i) diet and trophic relationships of selected seabird species, and (ii) anthropogenic pollutant levels, with the purpose of testing the hypothesis that guano (and subsequently uric acid) is a valid non-invasive sampling technique. Presented below are the main conclusions that have come out of this research.

Analysis of seabird guano and uric acid as an indicator of seabird dietary patterns

- This is one of the first studies to investigate the use of stable isotopes from seabird guano and extracted uric acid to infer foraging behaviour of seabirds, with the data acquired falling largely in the range of results reported for some tissues (e.g., particularly blood) in seabirds studies in the North Atlantic and North Sea.

- For most species the $\delta^{13}C$ and $\delta^{15}N$ signatures of uric acid were not significantly different from that of guano (<1‰ for $\delta^{13}C$ and 1.2‰ for $\delta^{15}N$) with a highly linear relationship, although there was some slight variation in some species. It is considered likely that these slight differences observed are mostly likely attributed to the free-living nature of seabirds which consume different prey components resulting in differential excretion of non-uric acid components.

- The study has demonstrated that stable isotopic analysis of guano and uric acid can be used as an alternative to tissue analysis when investigating dietary behaviour of seabirds to provide short-term dietary information (in the order of a few days). Sampling guano is a much less invasive process and therefore an attractive alternative for studying avian ecology in free-living seabirds. However, it is clear that further work needs to be undertaken to understand the basis of diet-tissue fractionation and the role of excreted fluids and solutes in isotopic fractionation if we are to fully realise the potential of stable isotope analysis in dietary studies.

Using C and N isotopes in understanding the foraging ecology and dietary patterns of Scottish seabirds during the breeding season

- The study has shown notable inter- and intra-specific differences in $\delta^{13}C$ and $\delta^{15}N$ signatures across species and breeding locations. These differences are primarily influenced by the feeding ecology of each species, although aspects of spatial variation play a key part in some colonies due to the differentiated availability of fishery resources. The study found no evidence to suggest that variability in baseline isotopic signatures had a significant effect on isotopic relationships.

- The results demonstrate that seabird species such as Northern gannets (*Morus bassanus*) and Northern fulmars (*Fulmarus glacialis*) have $\delta^{13}C$ and $\delta^{15}N$ signatures consistent with a full marine diet, while other species such as herring gulls (*Larus argentatus*), particularly form Ailsa Craig, are feeding on a more terrestrially dominated diet (Figure 5.1).

- Species such as black-legged kittiwakes (*Rissa tridactyla*), European shags (*Phalacrocorax aristotelis*) and Razorbills (*Alca torda*) appear to be feeding on a very specific diet, with little intra-specific variability between individuals, while others such as Great skuas (*Catharacta skua*), and Manx shearwaters (*Puffinus puffinus*) appear to be feeding across a much wide-ranging prey base. These results are consistent with the strong dependence of kittiwakes, razorbills and shags on a narrow range of small lipid-rich pelagic shoaling species, such as lesser sandeels and sprat. In contrast, great skuas exhibit wider variability as a result of greater diversity of foraging strategies between individuals.

- While inter-annual variation in the diet of seabirds has been widely reported in scientific literature, largely in response to fluctuations in food availability and changing energy requirements or reproductive constraints during the breeding season, the present study observed considerable isotopic overlap in black-legged kittiwakes from the Isle of May and Dunbar Harbour between sampling years (Figure 5.2), suggesting that individuals from these colonies were consuming

isotopically similar prey, taken largely from similar regions. There was evidence of some slight fluctuations in isotopic signatures with birds sampled in 2007 exhibiting enriched $\delta^{13}C$ and $\delta^{15}N$ signatures, which are likely to be a reflection of dietary changes at these colonies, although factors such as changes in foraging area, body condition, and nutritional stress which are also known to influence the isotopic signatures of seabirds should not be ignored.

- The study found limited evidence of intra-annual isotopic variability within the colonies, suggesting that birds were largely feeding on prey of similar isotopic signatures and in the same foraging regions throughout the breeding season; this is largely in agreement with published literature on kittiwake foraging behaviour at these colonies.

Trace elements in Scottish seabird populations

- The results reported in the present study for trace element concentrations in guano, with the exception of lead (Pb), are largely within the ranges reported in seabirds studies in Antarctica, Arctic and Southern China Sea, despite different geographical locations.

- While the literature clearly shows that high metal levels are consistently reported in some seabird species, the elevated Pb levels reported in the present study are much higher than those previously reported for guano. Indeed, it is clear that the levels found in this study appear to be higher than 10ug g^{-1} (dry weight) as described in guano from other bird species, suggesting that the majority of species in this study have a high Pb dietary burden, with both herring gulls (36ppm) and gannets (40ppm) showing highly elevated levels which are clearly in the region of physiological effects. However, in the absence of available literature about the absorption, accumulation and excretion of Pb in marine birds, it is clear that further information is required to understand whether these results are a true reflection of Pb burden in these seabirds.

- The study demonstrated notably higher trace element concentrations in gannets and herring gulls compared to the other species studied. A number of factors were proposed for these differences, but it is likely that they can be attributed to the species highly diverse diets, and in the case of gannets, their higher trophic status which mean that they are consuming prey items with higher trace element burdens. However, the study clearly pointed out that other contributing factors must also have an impact.

- The study reported some notable low concentrations of trace elements, with great skuas from Foula exhibiting the lowest levels across all elements with the exception of As and Se. As a relatively pristine environment it can be expected that species from Foula would have reduced element levels compared with other areas, although it does not explain the consistently low levels seen for great skuas as opposed to other species sampled at the site. Great skuas from Foula are known to feed on a mixture of sandeels, gadids and other seabirds, therefore dietary habitats could not be the only explanation for this observed pattern, other factors including the birds physiological and biochemical processes could be potential contributors.

- Evidence of intra-specific differences between species was found for some trace elements in great skuas and kittiwakes that can largely be explained by dietary behaviour between colonies in the former, although anthropogenic factors such as vessel movement cannot be ruled out in the latter.

- In conclusion, the results from the study show that there are both similarities and differences in trace element concentrations both within and between species that can largely be attributed to dietary variation, although other factors including anthropogenic activities can potentially contribute to this variability in specific locations. With knowledge of the sources and controls on metal variability in diets and bodily accumulation such data derived from seabird guano can provide a potentially useful bio-monitor of trace element concentrations in the wider marine environment.

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