UNIVERSITY OF SOUTHAMPTON

Faculty of Engineering, Science and Mathematics
School of Civil Engineering and the Environment

Heavy Metal Pollution and Black-headed Gull (*Larus ridibundus* L.) Breeding Ecology

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ABSTRACT

FACULTY OF ENGINEERING, SCIENCE AND MATHEMATICS
SCHOOL OF CIVIL ENGINEERING AND THE ENVIRONMENT

Doctor of Philosophy

TITLE OF THESIS: HEAVY METAL POLLUTION AND BLACK-HEADED GULL (*LARUS RIDIBUNDUS* L.) BREEDING ECOLOGY

Author: Kirsty Louise Pickard

Heavy metals in air, soil and water are a global problem and present a growing threat to the environment. These metals may have profound consequences for birds and can cause a number of sub-lethal effects, such as decreased reproductive success. The concentrations of selected heavy metals (As, Cd. Co, Cu, Fe, Pb, Mn, Ni, V, Zn) and Se in eggs and feathers from populations of black-headed gulls (*Larus ridibundus* L.) located on different colonies in the UK, which have different characteristics and are subject to different sources, types and degrees of pollution, were examined.

Concentrations of As, Cu, Pb, Ni, Se and V measured in black-headed gull eggs were consistently high relative to those reported in previous field studies with other gull species. However, no significant effect was observed on the egg characteristics in terms of egg size and dimensions, shell thickness and index as a result of concentrations of metals measured in this study. Concentrations of Co, Fe and Ni were significantly negatively correlated with yolk:albumen ratio in the egg. The usefulness of sampling eggs to provide a reflection of local contamination has been demonstrated, with concentrations related to local sources of metal pollution and site differences reflected in sediment concentrations from previous studies. The importance of taking into account diffuse and historical pollution in addition to point source discharges has also been highlighted.

As, Fe, Mn, Pb, Se, V and Zn were found at significantly higher concentrations in egg contents than egg shell, and Cd, Co and Ni concentrations were higher in shell than contents. Cu was distributed approximately equally. Within the egg contents, concentrations of As, Cu, Se and V were higher in the albumen than in the yolk, and Co, Fe, Mn, Ni, Pb and Zn concentrations were higher in the yolk than the albumen. Cd was found mainly in the shell and concentrations in egg contents were largely undetectable.

Comparisons were made between a colony subject to high-level commercial egg harvesting and an un-harvested site, and between pre- and post-harvesting eggs on the harvested site. Post-collection eggs were found to be of significantly lower quality than the pre-collection eggs and the eggs from the uncollected site, as indicated by yolk:albumen ratio. Concentration of metals in eggs as a result of relaying forced by commercial harvesting has been demonstrated, with concentrations of Co, Fe and Ni significantly higher in post-collection eggs compared to pre-collection eggs. Average nesting density was significantly lower on the collected colony than the uncollected colony. No effect on egg size was found as a result of changes in nesting density.

Concentrations of metals in black-headed gull chick down were measured and compared to egg data in order to assess the usefulness of feathers as a tool for non-destructive monitoring of metal pollution. The results suggest that feathers may be good indicators for As and Zn, and possibly also for Mn and Ni. However, the sample masses were very small and for a number of metals concentrations were largely undetectable using the analytical equipment available in this study. Future work with larger samples of down would be prudent to further examine the use of chick down to provide an indication of the level of pollution to which birds are exposed. The importance of using appropriate washing procedures to remove exogenous contamination of feathers to assess internal concentrations has been demonstrated.

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DECLARATION OF AUTHORSHIP

I, **Kirsty Louise Pickard**

declare that the thesis entitled

Heavy Metal Pollution and Avian Population Ecology in the UK

and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;
- where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
- where I have consulted the published work of others, this is always clearly attributed;
- where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
- I have acknowledged all main sources of help;
- where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
- none of this work has been published before submission.

Signed: ……..

Date:…….

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AONB	Area of Outstanding Natural Beauty
ATSDR	Agency for Toxic Substances and Disease Registry
BTO	British Trust for Ornithology
Defra	Department for Environment, Food and Rural Affairs
DMA	Dimethyl arsenic
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
EA	Environment Agency
EC	European Commission
EHC	Environmental Health Criteria
EU	European Union
HSDB	Hazardous Substances Data Bank
HR ICP-MS	High Resolution Inductively Coupled Plasma Mass Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
K-S	Kolmogorov-Smirnov
LD_{50}	Lethal Dose, 50% (median lethal dose)
LNR	Local Nature Reserve
MARS	Microwave Accelerated Reaction System
MMA	Monomethyl arsenic
NAS	National Academy of Sciences (US)
NNR	National Nature Reserve
NRCC	Natural Resource Council Canada
PFA	Perfluoroalkoxy resin
RSPB	Royal Society for the Protection of Birds
SAC	Special Area of Conservation
SI	Shell Index
SPA	Special Protection Area
SSSI	Site of Special Scientific Interest
STW	Sewage Treatment Works

CHAPTER 1. GENERAL INTRODUCTION

Metals are naturally occurring elements that become contaminants when human activity raises their environmental concentration above natural levels. Toxic heavy metals in air, soil, and water are global problems that are a growing threat to the environment. Whether in the terrestrial or aquatic environment, metals can be transported by several processes, governed by the chemical nature of metals, soil and sediment particles, and the pH of the surrounding environment.

Although heavy metal pollution is associated with areas of intensive industry, pollution from diffuse sources such as agricultural land and urban areas is also a significant source of heavy metals to the environment, and pollution arising from roadways and automobiles is now considered to be one of the largest sources (Cicchella *et al.*, 2008). Zinc, copper and lead are three of the most common heavy metals released from road travel, accounting for at least 90% of the total metals in road runoff (NVSWC, 2005). Lead concentrations, however, have consistently decreased since the use of leaded petrol was discontinued. Smaller amounts of many other metals, such as nickel and cadmium, are also found in road runoff and exhaust. Metals can also enter the environment via natural sources, such as weathering of rocks and soils. Table 1-1 provides and overview of sources of metals and the environments affected. A more in-depth analysis of the sources, transport and fate of heavy metals and selenium is provided in Chapters 4 and 5.

Table 1-1 Common sources of metal pollution

Source	Particular metals associated	Environment affected
Natural weathering of rocks and soils	As, Cd, Fe, Pb, Ni, Se, V, Zn	All
Runoff from agricultural land	As, Cd, Co, Cu, Fe, Mn, Se, V, Zn	All
Runoff from urban areas and roads	Cd, Cu, Pb, Ni, V, Zn	All
Industry	Numerous - commonly Cd, Cu,Hg, Pb; specific metals dependant on industry type	All
Boats and shipping	Cu, Sn, Zn	Aquatic

The metals examined in this study are arsenic, cadmium, cobalt, copper, iron, lead, manganese, nickel, selenium, vanadium and zinc, based on those that are perceived to be environmentally

and toxicologically significant (included on the EU Dangerous Substances Directive (76/464/EEC) and the EU Water Framework Directive (2000/60/EC)), and those for which data regarding the effects on avian breeding ecology is limited. Although selenium is a semi-metal rather than a heavy metal, it is included on the EU Directives above and is often considered alongside heavy metals because it has many metal-like characteristics and is released from many of the same sources as heavy metals (Eisler, 1985b; WHO, 1986; see also Table 1-1).

It is widely recognised that coastal areas are among the most sensitive zones around the world, and the marine environment is faced with a number of increasingly severe threats, including loss or degradation of biodiversity and changes in its structures, loss of habitats and the impacts of climate change (EC, 2006). Contamination of the marine ecosystem with dangerous substances has been one of the main threats and concerns in recent years, and the volume of literature now available on various aspects of marine pollution is extensive (for example: Law *et al.*, 1997; Hall & Anderson, 1999; Boxall *et al.*, 2000; Tanabe, 2002; Braune & Simon, 2004). World-wide use of chemicals is constantly increasing, be it in industry, on farms or in homes, and pollution may be considered as the main, most widespread and most dangerous manifestation of anthropogenic impact on the aquatic environment. Marine pollution frequently originates on land, entering the sea via rivers and pipelines, and it is estimated that land-based activities account for around 80% of marine pollution (EC, 2006). However, inputs from sources at sea such as ships and offshore platforms, and from atmospheric sources, also contribute to the pollution of marine waters (Spencer & MacLeod, 2002). Pollutants enter the marine environment in three main ways: 1. direct discharge of effluents and solid wastes into the seas and oceans, from point sources such as industrial discharge, municipal waste discharge, coastal sewage, sewage outfalls and so on, 2. from non-point sources (diffuse pollution, arising from many locations) such as land runoff in the coastal zone, mainly with rivers, and 3. long-range atmospheric transport and wet and dry deposition. The latter type of pollution has increased in recent decades as many chemicals from power plants and industries are transported to all regions, including the Arctic and the Antarctic (Houghton *et al.*, 1992). The relative contribution of each of these pathways into the combined pollution input into a system will be different for different substances, and in different situations (Patin, 1999).

Because the main origins of marine pollution are on land, coastal waters tend to be more polluted than open seas, with estuaries and harbours being among the worst affected. The aquatic environment is particularly at risk because pollutants can spread rapidly from the source of the pollution, and over large distances, in the water environment (Patin, 1999; Burger & Gochfeld, 2002). After entering the marine environment, contaminants are usually diluted and

widely dispersed; however, many contaminants are adsorbed onto particulate matter, which leads to elevated concentrations in areas where this material settles and, consequently, sediments may act as long-term stores for pollutants in the environment (Spencer & MacLeod, 2002).

1.1 Seabirds as monitors of pollution

Marine birds may be defined as those birds living in and utilising resources from the marine environment, which includes coastal areas, islands, estuaries, wetlands and oceanic islands. Seabirds are a subset of marine birds and include those that feed at sea, either nearshore or offshore, although not always exclusively, and are equally at home on land, in the air, and in the water (Schreiber & Burger, 2002). As one of the ultimate examples of colonial living, over 95% of seabirds are colonial, with colonies often consisting of several species and ranging from a few pairs to many thousands or even millions (Schreiber & Burger, 2002). Colonial species tend to have easily detected, visible, and long-established breeding sites at which large amounts of data may be gathered, making them particularly easy to study (Furness *et al.*, 1993).

Because they spend most of their time in aquatic environments where they are exposed to pollutants by external contact, inhalation, and ingestion of food and water, seabirds are exposed to a wide range of chemicals and other forms of pollution (Burger & Gochfeld, 2002; see Figure 1-1). The major groups of pollutants of concern are metals, chlorinated hydrocarbons, petroleum products, plastic particles, and artefacts (man-made debris such as plastics, polystyrene and so on), although more recently attention has focused on a much wider range of industrial and agricultural compounds which may be bioactive, including endocrine-disrupting chemicals. Birds are exposed to metals mainly via their food, water, respiratory exposure to airborne contaminants and through the cleaning of their feathers (Goede & de Bruin, 1984; Dauwe *et al.*, 2004). For seabirds, ingestion of food and water are the main routes of exposure (Burger & Gochfeld, 2004).

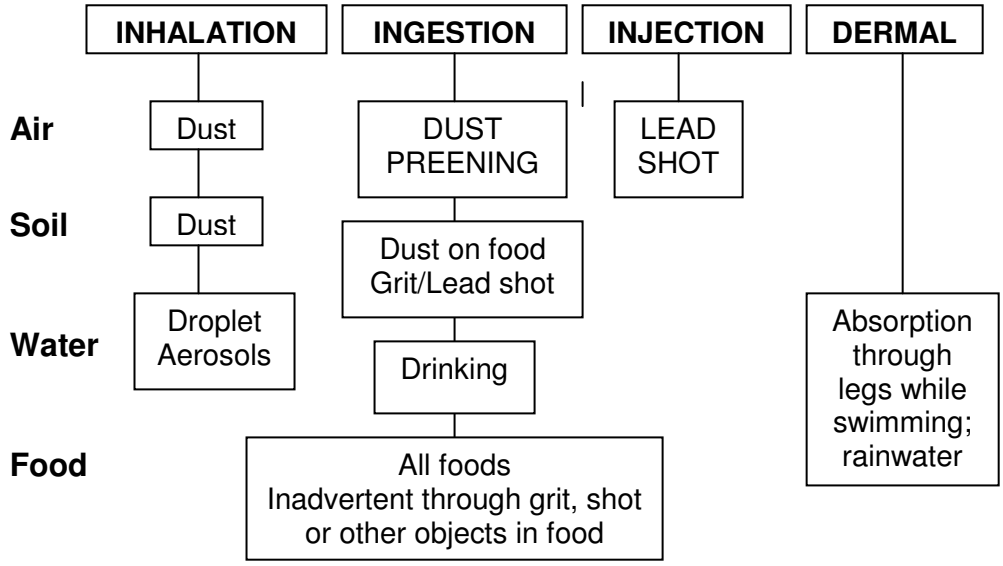


Figure 1-1 Pathways of exposure for seabirds in air, soil, water and food (adapted from Burger & Gochfeld, 2002)

The effects of certain pollutants (particularly organochlorines and mercury) on avian population ecology have been widely documented (for example: Blus *et al.*, 1979; Custer *et al.*, 1983; Pain *et al.*, 1999; Thyen *et al.*, 2000; Connell *et al.*, 2003); however, the impacts of some metals and organic contaminants have received far less attention. Heavy metals may have profound consequences for birds, even at concentrations insufficient to cause death or other acute effects, and cause increased susceptibility to disease or other stresses, changes to normal behaviour patterns and decreased reproductive success (Heinz, 1974; Scheuhammer, 1987; Burger & Gochfeld, 1995b; Heinz *et al.*, 1999). Table 1-2 provides a summary of the sub-lethal effects associated with elevated concentrations of metals in birds.

Table 1-2 Sub-lethal effects 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 and specific nature of a pollutant determines whether it causes an effect; since different families of seabirds, and different species within these families, have different life-cycles, behaviour, ecologies, and habitat uses, their respective vulnerability also varies (Burger & Gochfeld, 2002). Susceptibility may also vary with age, reproductive stage and gender. As well as exhibiting direct effects, heavy metal pollution may also affect bird populations through effects on the abundance of prey organisms (Bryan & Langston, 1992). Birds, particularly those species at the top of their food chain, are extremely valuable as biological monitors of environmental change, and may act as 'sentinel' species for the harmful effects of specific pollutants. Sentinel species are usually chosen because they are particularly sensitive to a specific pollutant and thus provide the earliest warning of pollution in an ecosystem (Furness *et al.*, 1993). Concentrations of contaminants in seabirds can be examined both as an indication of potential harm to the seabirds themselves, and as an indication of coastal and marine pollution in general (Walsh, 1990; Peakall, 1992; Furness, 1993; Furness & Camphuysen, 1997). Seabirds have been used to assess pollution over local, regional or wide-scale geographical areas as well as to determine whether concentrations of contaminants have changed over time (Walsh, 1990). For example, seabirds have been used as biomonitors of serious organochlorine pollution in the

North Sea, the effects of which were first noticed as a result of the effect on local seabird populations (Furness & Camphuysen, 1997).

There are a number of particular advantages to using birds as monitors of pollution, the main benefits being simply that they are relatively easy to study and large amounts of data have already been gathered for bird populations, and much research carried out into their ecology and behaviour (Furness *et al.*, 1993). With the ecology of most bird species being quite well known and their classification and systematics well established, there is little risk of monitoring being confounded by uncertainties regarding the identities of, or relationships between, the species being studied (Furness *et al.*, 1993). Seabirds, in particular, make excellent bioindicators because they are sensitive to chemical and radiological hazards and are widespread over the world in coastal and marine habitats, where pollution is often significant and where contaminants are transported rapidly through aquatic systems and within food chains (Burger & Gochfeld, 2002). Seabirds also feed at the upper trophic levels of ecosystems where they can be exposed to relatively high concentrations of contaminants in their prey (Burger & Gochfeld, 2002) and so can provide information on the extent of contamination in the whole food chain. This, combined with the fact that they are long-lived, makes seabirds particularly vulnerable to contaminants and susceptible to the effects of bioaccumulation. In addition, being at the top of the food chain means seabirds may reflect pollutant hazards to humans better than most invertebrates and other organisms lower in the food chain, but also means that they may be sensitive to many diverse factors affecting the food chain (Furness *et al.*, 1993), such as seasonal temperature and weather conditions.

Although the mobility of birds can allow monitoring over a broad spatial scale (the breadth depending on the species chosen), migratory habits can render birds much less suitable as biomonitors because individuals may differ in their migrations to an uncertain extent, and thus it can be difficult to determine the spatial scale they represent (Furness *et al.*, 1993) and exactly when and where exposure occurred. Populations of different origins pass through the same place at different times of year, and, in the case of pollutant monitoring, pollutants will be picked up from a wide and often ill-defined area (Furness, 1993). This problem can be minimised by using sedentary species or young birds that have not yet fledged and have obtained all their food from their parents which, in most cases, have obtained it from the local area prior to breeding (Burger & Gochfeld, 2004). Most birds in tropical and temperate regions spend many weeks on the breeding grounds before laying, with the breeding females acquiring sufficient resources locally to produce eggs (Burger, 2002). In a study with black-headed gulls (*Larus ridibundus*) - the species of interest in this study - it has been demonstrated that the

maximum foraging distance during breeding is 18.5 km (Gorke & Brandl, 1986). The flight radius in this study was less than 5 km from the nesting site when the nest contained eggs or newly hatched chicks, with the parent foraging only one or two times a day and spending less than one hour (per flight) away from the nest. This behaviour appears to be induced by eggs or nestlings, with one gull in the study, having lost its clutch, immediately flying greater distances to forage (between 9 and 14 km). It is suggested by Gorke & Brandl (1986) that the constraints to the foraging radius of parent birds are relaxed as the nestlings grow and the foraging radius can be increased as time required to guard the nest is decreased. This is attributed to the fact that, as the chicks grow, they are more capable of regulating heat themselves, and are less likely to be killed by neighbouring birds. These studies demonstrate that breeding birds feed in a reasonably localised area prior to and during breeding and raising chicks, and are unlikely to have obtained resources from a distance greater than 18.5 km from the nesting site during this period.

Although some seabird populations are threatened or endangered through habitat loss, exploitation, overfishing and other anthropogenic impacts (Croxall *et al.*, 1984), populations of many species are robust and the collecting of limited individuals does not pose a conservation problem (Burger & Gochfeld, 2002). However, some species, top predators in particular, are extremely scarce and thus it is not usually possible to take large samples of birds or eggs. In the case of tissue sampling, this means that tissues of adults are often obtained from birds found dead, including those that have starved. Starvation causes mobilisation of fat reserves, which often contain the highest concentrations of a contaminant (for example organochlorines which, being lipophilic, readily partition to the body fat) and hence the depletion of fat reserves in a starving bird may lead to an increase in the concentration of contaminants in soft tissues (Furness & Camphuysen, 1997), particularly the liver (Bogan & Newton, 1977). This can lead to a great deal of variability between samples, and it is important to bear this in mind when interpreting and comparing results of different studies. It is also important to take into account the season and the age, sex and diet of the birds, as the lipid store varies with all these factors, and hence concentrations of some contaminants may also be variable (Anderson & Hickey, 1976; Clark *et al.*, 1987).

Practical and ethical reasons inhibit the sacrifice of free-living animals; as a result, new methods for non-destructive biomonitoring are always being developed (Furness, 1993). Avian biomonitors have been successfully used with the following aims: to indicate temporal and spatial trends in chemical pollution in terrestrial and aquatic ecosystems; to monitor marine pollution; to detect diverse environmental changes such as habitat alteration or fragmentation,

and climate change by monitoring bird populations (such as abundance, distribution and demography; Becker, 2003). However, despite their undoubted advantages as biomonitors, birds are not used as often or as effectively as they could be. Birds have several methods of ridding the body of contaminants, including normal excretion via faeces and through transfer to eggs (Burger, 1994), deposition in the uropygial gland, salt gland (Burger & Gochfeld, 1985; Goede & de Bruin, 1986), and feathers (Braune, 1987; Braune & Gaskin, 1987a; Braune & Gaskin, 1987b; Lewis & Furness, 1991; Burger *et al.*, 1992). The use of eggs or feathers as indicators is a potentially very useful non-invasive method of obtaining an indication of tissue concentrations of a number of contaminants in birds. However, while many biomonitoring studies with birds have focused on organic pollutants and certain heavy metals, such as mercury, in eggs (Parslow & Jeffries, 1977; Barrett *et al.*, 1985; Burger & Gochfeld, 1991; Burger & Gochfeld, 1993; Burger & Gochfeld, 1995a; Burger & Gochfeld, 1996), investigation into the use of eggs to provide an indication of local pollution for other metals has been limited, and further research is required in this area. Feathers may also be used as effective biomonitoring tools, and have the advantage that they can be collected irrespective of season, age or sex. In addition, feathers provide a truly non-destructive method of sampling, and this technique could therefore be valuable as a non-destructive biomonitoring tool for endangered species. Studies have been carried out to investigate the link between heavy metal concentrations in feathers and the concentrations in internal tissues (Goede & de Bruin, 1986; Burger, 1993; Lewis *et al.*, 1993); however results have been inconclusive and, in some cases, conflicting, and further study is required to assess the suitability of feathers as biomonitors of metals.

1.2 Eggs as indicators of pollution

The egg supplies the embryo with all the necessary nutrients for growth, and provides the optimum environment for development (Perrins, 1996). Each of the three main egg components has an important role in the development of the embryo: the yolk provides the majority of the necessary nutrients for the developing embryo, the albumen also provides nourishment, as well as the structural support needed for the attachment of two shell membranes during development of the egg, and the shell provides protection, minerals (primarily calcium) and regulates gas and water exchange (Romanoff & Romanoff, 1949; Perrins, 1996; Karlsson & Lilja, 2008).

Adult females can transfer pollutants to eggs as a method of eliminating them from the body (Wiemeyer *et al.*, 1984; Tanabe *et al.*, 1986; Burger, 1994; Bargar *et al.*, 2001; Mora, 2003); thus determination of contaminants in eggs can give a good indication of adult exposure to those

chemicals (Burger, 1994). Eggs are a good indicator of local exposure to contaminants since, as previously mentioned, the breeding females of most bird species spend the weeks prior to laying acquiring resources to produce eggs (and thus any associated contaminants) from an area local to the breeding ground (Burger, 2002). The use of eggs for contaminant analysis eliminates many of the problems associated with sampling tissues. Eggs have a highly consistent composition, unlike the liver (the traditionally sampled tissue) which changes significantly in size and composition during both the day and the year (Furness *et al.*, 1993). In addition, eggs are produced by a clearly identified segment of the population - adult females – although in some ways this can be a disadvantage as it excludes a large proportion of a population. Egg sampling takes very little time and the eggs themselves are easy to handle and easier to store than dead birds or tissues. Eggs can also be taken with little disruption and egg sampling places less of a strain on the population than the sampling of adults, particularly if only one egg is sampled from each clutch (Potts, 1968).

In spite of the advantages over internal tissues, as a monitoring unit the egg also comes with its own set of problems. The fact that pollutants in eggs usually represent pollutant uptake in a short period before the egg is laid means that they cannot be used to examine pollutant burdens acquired at other times of the year (Furness *et al.*, 1993); conversely, concentrations may sometimes be affected by pollutant burdens built up over the long term which can confound interpretation of results. Concentrations of pollutants have also been shown to vary through the clutch sequence, with the general observation being that concentrations are higher in the last laid egg than in the first laid (Becker, 1989). When sampling for monitoring studies, eggs should therefore be taken at the same position in the laying order from each of the clutches. In this study, eggs were taken from nests containing only one egg which ensured that the eggs analysed were the first laid in the clutch.

Regular egg collection from populations of many wild bird species is unacceptable (and often illegal) for conservation reasons and hence analyses are frequently carried out on deserted or addled eggs that remain in the nest after chicks have hatched (Burger, 1994; Gochfeld & Burger, 1998; Pain *et al.*, 1999; Ormerod *et al.*, 2000; Burger *et al.*, 2004; Herzke *et al.*, 2005; Ikemoto *et al.*, 2005; Jaspers *et al.*, 2005). Addled or deserted eggs may not have pollutant concentrations typical of the whole population as they are likely to have been produced by birds that are young, of poor quality or those that have been particularly affected by pollutants (Furness *et al.*, 1993). Eggs lose water during incubation and thus eggs collected from nests long after being due to hatch tend to be severely dehydrated and may be subject to bacterial infection (Furness *et al.*, 1993). Similarly, much water can be lost from eggs during storage,

including freezing, and contaminant concentrations may therefore be affected; this problem can be avoided if dry weights are used when presenting contaminant concentrations. Wet-weight comparisons between eggs of different species are also questionable, as the water content tends to be highest in species hatching altricial (i.e. naked, poorly developed) chicks (Romanoff & Romanoff, 1949).

Eggs can also be used as an indication of reproductive health and breeding success. Studies have shown a positive relationship between egg size and posthatching survival and growth in birds (Parsons, 1970; Lundberg & Väisänen, 1979; Bolton, 1991; Hipfner & Gaston, 1999), demonstrating the developmental advantage of larger eggs. The size of eggs laid by a bird is variable among species, and egg size relative to body size has been suggested as a possible reason for interspecies variability in excretion of contaminants (Lemmetyinen *et al.*, 1982). In addition, the number of eggs laid in a clutch may also affect the excretion of a chemical, as a smaller clutch provides less opportunity to excrete chemicals, and thus a greater percentage of maternal body burdens may be excreted into the eggs (Bargar *et al.*, 2001). The quality of the parents can contribute as much to the quality of the offspring as the habitat (Blomqvist *et al.*, 1997); however, where eggs produced in an area are consistently small, this could be an indication of poor habitat (Fair & Myers, 2002).

1.2.1 Effects of pollution on eggshell thickness

The shell of an egg contributes to successful embryogenesis in many ways: through protection from crushing during incubation, providing resistance to entry of pathogens, controlling gas and water exchange, and providing the embryo with minerals, primarily calcium, which is required for the development of the skeleton, muscles and brain (Tuan *et al.*, 1991; Blom & Lilja, 2004; Karlsson & Lilja, 2008). The impacts of eggshell thickness on breeding success are discussed in detail in Chapter 2, Section 2.1.2.

Eggshell thinning as a result of organochlorine pollution is well-documented, and the effects of thinner shells on the reproductive success of birds are well known. Thinning of the shells of bird eggs was first documented just over 40 years ago (Ratcliffe, 1967). By comparing fresh samples of eggs with museum specimens, Ratcliffe demonstrated that shell thinning in British birds of prey had begun at the same time as the first widespread use of the pesticide dichlorodiphenyltrichloroethane (DDT) in 1947 (Ratcliffe, 1967; Ratcliffe, 1970). Figure 1-2 illustrates the sudden and unprecedented decrease in the eggshell index of the sparrowhawk (*Accipiter nisus*) during 1946-1950.

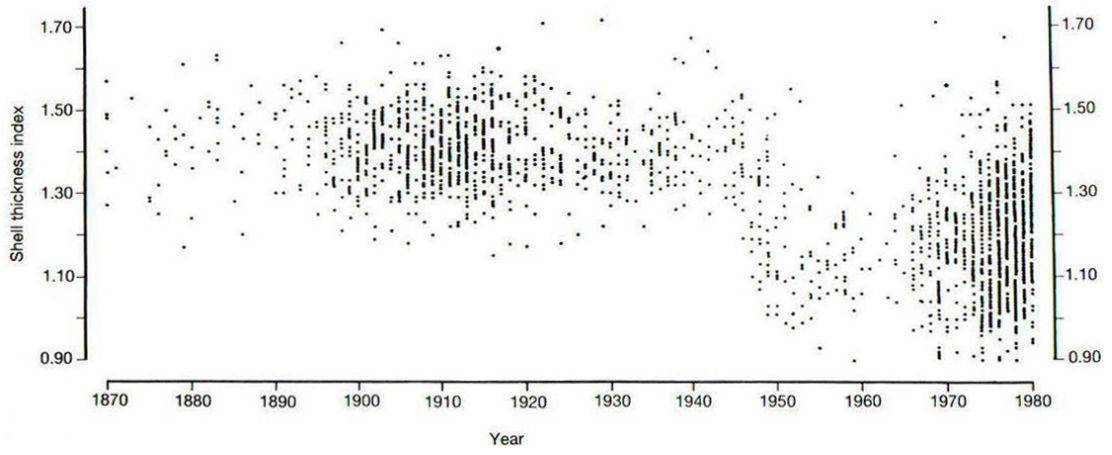


Figure 1-2 Shell thickness index of British sparrowhawks, 1870-1980 (Furness, 1993, after Newton, 1986)

It later became clear that the degree of shell thinning in birds could be related to residues of dichlorodiphenyldichloroethylene (DDE), a metabolite of DDT, in the egg contents and laying female; however, other organochlorines such as polychlorinated biphenyls (PCBs; Forsyth *et al.*, 1994) have also been suggested as having the same effect, as well as some heavy metals (Lundholm, 1987; Lundholm, 1995).

In general, the impacts of metals on eggshell thickness have received far less attention than organic substances such as organochlorines and pesticides. While the impacts of some metals on eggshell thickness have been reported, no information is available for others. Of the metals investigated in this study, literature data have been found suggesting that arsenic, cadmium, lead and zinc may lead to shell thinning in bird eggs (Haegele & Tucker, 1974; Edens *et al.*, 1976; Hussein *et al.*, 1988; Furness, 1996). However, studies are few and, in some cases, offer conflicting results, and it is therefore difficult to draw definite conclusions. No studies reporting the effects of cobalt, copper, iron, manganese, nickel, selenium and vanadium on eggshell thickness could be found. The lack of conclusive data or, in some cases, any data at all, regarding the impacts of heavy metals and selenium on shell thickness means that this is an area of research that requires further investigation. Chapter 5 of this thesis attempts to relate concentrations of heavy metals and the associated semi-metal selenium in black-headed gull eggs to effects on the quality of the shells.

1.2.2 Partitioning of metals in the egg

The partitioning of different metals between eggshells and egg contents has been examined by a number of authors (Burger, 1994; Morera *et al.*, 1997; Mora, 2003; Agusa *et al.*, 2005; Ikemoto *et al.*, 2005; Lam *et al.*, 2005). Results for most metals are conflicting, with some authors reporting a metal to be partitioned more in the eggshell than contents, others reporting the reverse, and yet more studies describing a fairly even distribution between shell and contents. Detail of the studies can be found in Chapter 5, Section 5.1.3. In all of these studies a proportion of most metals was found to accumulate in the eggshell. In addition to differences in concentrations between eggshells and egg contents, different metals may bind to different components of the egg contents. However, only one study could be found reporting partitioning of metals between egg yolk and albumen: Magat and Sell (1979) found that selenium binds preferentially to albumen, rather than yolk. No studies could be found reporting partitioning of any of the other metals of interest in this study between egg yolk and albumen. Many previous studies examining metal concentrations in eggs have analysed only the egg contents and neglected to analyse the eggshell (for example: Hernández *et al.*, 1988; González & Hiraldo, 1988; Burger & Gochfeld, 1991; Baranowska *et al.*, 2005), and analysis of egg contents has examined homogenised contents rather than separate components. For metals such as cadmium, for which there is evidence that it partitioning may be mainly to the eggshell and concentrations in egg contents negligible (Dauwe *et al.*, 2005), previous studies that have reported low or undetectable concentrations in eggs may have done so because the authors analysed only the egg contents and did not analyse the eggshell (for example: Sell, 1975; Scheuhammer, 1987; Burger & Gochfeld, 1993; Braune & Simon, 2004). It may be prudent for future studies examining metals in egg contents to adopt a more targeted approach, focusing analysis on a particular component of the egg. For example, if a metal is present almost entirely in the yolk, when looking at low concentrations analysis of homogenised egg contents will only serve to dilute the concentrations of these metals in the sample, and analysis of yolk rather than homogenised egg contents might provide the best assessment of concentrations on the egg contents. Ideally, in order to obtain accurate results for total concentrations in the egg, all three egg components should be examined separately, and the results combined to provide an accurate 'total' egg concentration.

The present study aims to address the gaps in the knowledge regarding metal partitioning in eggs, and will examine partitioning of arsenic, cadmium, cobalt, copper, iron, lead, manganese, nickel, selenium, vanadium and zinc between egg shell, yolk and albumen (Chapter 5).

1.3 Feathers as indicators of pollution

Birds use feathers as a means of both storing essential elements and eliminating contaminants, incorporating substances in the keratin structure during the short period of feather growth when the feather is connected with the blood stream through small blood vessels (Goede & de Bruin, 1984; Burger, 1993; Dauwe *et al.*, 2000). Thus, feathers can be effective indicators of contamination in internal tissues, and indeed have been used in many studies as an indication of contamination of a number of metals, in particular mercury (see Section 6.1). Samples of feathers can be taken from dead specimens but also from live birds with virtually no effect on the birds sampled (Burger, 1993), especially if body feathers are taken rather than flight feathers, enabling a much larger sample of feathers to be taken. In addition, feathers can be easily stored without being frozen, meaning sampling from remote populations is much simpler than with tissue collections (Furness *et al.*, 1993). In many ways, feathers are thus an ideal non-destructive method for measuring contaminant load in birds.

However, there are a number of potential disadvantages to the use of feathers as a measure of contaminant load in birds. Variations in the age composition of a sample of seabirds could potentially contribute to apparent differences in concentrations of contaminants in feathers (and other tissues) between species, populations or years (Gochfeld *et al.*, 1996; Burger & Gochfeld, 2000b). In a review of studies of metals in feathers, Burger (1993) reports that adults had significantly higher concentrations than young for mercury (20 of 21 studies), lead (4 of 7), cadmium (3 of 5) and manganese (5 of 5). The same author conducted investigations into concentrations of metals in the feathers of adult and juvenile Franklin's gulls (*Larus pipixcan*; Burger, 1996). For most comparisons where age differences were observed, feathers taken from adults had significantly higher concentrations of heavy metals than those taken from young. Several other studies also report a similar link between age and feather concentrations of metals (Gochfeld *et al.*, 1996; Burger & Gochfeld, 2000b). Concentrations in adult feathers might be expected to be higher than those of fledglings because the adults have had several years to bioaccumulate metals in their internal tissues. In addition, fledgling feathers represent contamination acquired almost entirely on the breeding grounds and from development in the egg, while adult feathers collected on the breeding ground were grown on the wintering grounds and reflect contamination also obtained there. Chicks are continually exposed to metals acquired from the food brought by the parents, in addition to exposure from contact with the mother prior to hatching, contact with parents and other birds after hatching, exposure to excrement, and from external sources via wet or dry deposition. As a result, nearly fully-grown

chicks, which have had several months to sequester metals in down feathers, may have higher concentrations of metals in tissues than recently-hatched chicks. Variation might also arise as a result of the differences in foods consumed by adults during the breeding season, or different-sized food items. In order to reduce variation due to age, it would be most sensible to take feathers from birds of a known similar age (Burger, 1995).

The limited data for the relationship between gender and metal concentrations in feathers are conflicting, and overall would suggest that gender differences in metal concentrations in feathers are not significant. For mercury, a review of studies by Burger (1993) reports that significant differences in mercury concentrations with gender were found in only two of eight studies. The results from the two studies were conflicting, with higher concentrations found in females for great blue herons (*Ardea herodias*; Hoffman & Curnow, 1979), and higher concentrations in males for Bonaparte's gull (*Larus philadelphia*; Braune & Gaskin, 1987a). The latter difference was found only in post-moult (i.e. post-laying) feathers, suggesting that the lower concentrations in the females at this stage may be a result of elimination of mercury via egg laying. Gender-related differences in concentrations of lead, cadmium, mercury, manganese, chromium and selenium in the feathers (and other tissues) of laughing gulls (*Larus atricilla*) were examined by Gochfeld *et al*. (1996). Higher concentrations of lead were found in the feathers of males than in females. No differences were observed for cadmium, mercury, manganese, chromium or selenium in feathers. Burger and Gochfeld (1992b) examined gender differences in cadmium, lead, selenium, chromium, manganese and copper in feathers of black skimmers (*Rynchops niger*). The authors found that females had higher concentrations of lead and cadmium than males, but no differences were observed for selenium, chromium, manganese or copper. It would seem that, in general, gender differences are unlikely to affect the concentrations of metals in seabird feathers, and thus it would not be considered necessary to sex individual birds from which feathers are to be sampled.

When considering contaminant loads in feathers from fledged birds, it is important to sample the same feather type, and to understand both the timing and pattern of moult and where birds were at the time of moult. During the moult, concentrations of some heavy metals in internal tissues drop as they are sequestered into feathers (Dauwe *et al.*, 2003) and, when the moult is completed, concentrations of some heavy metals rise in internal tissues until the next moult, when the process is repeated (Braune & Gaskin, 1987a). As a result, the internal concentration during the moult may not be constant, and may be high at the beginning and lower at the end of the moult (Dauwe *et al*., 2003). This effect has been noted for mercury by a number of authors (Appelquist *et al.*, 1984; Furness *et al.*, 1986; Braune, 1987; Burger & Gochfeld, 2000b; Dauwe

et al., 2003), with mercury concentrations tending to decrease linearly along such feather sequences as the primaries, corresponding to the order in which feathers have been dropped and renewed. This effect suggests that mercury concentrations in feathers adequately reflect concentrations in the blood during feather growth and are only slightly, if at all, affected by external contamination (Dauwe *et al*., 2003). For metals other than mercury, results are conflicting. Dauwe *et al*. (2003) found copper and zinc concentrations were significantly positively correlated with the moulting sequence in the little owl (*Athene noctua*); in the same study, however, copper and zinc concentrations were significantly negatively correlated or not correlated with the moult in the sparrowhawk (*A. nisus*). For most other elements the concentration in the primaries was either significantly negatively correlated or not correlated. Metal concentrations in feathers have also shown variation with the body location and type of feathers sampled (Goede & de Bruin, 1984; Walsh, 1990; Altmeyer *et al.*, 1991; Lewis & Furness, 1991; Burger & Gochfeld, 1997; Dauwe *et al.*, 2003; Muralidharan *et al.*, 2004). However, it would appear that, in most cases, a large part of the metal concentration differences measured between different feathers results from external deposition. Thus the relationship is not as simple as previous suggestions that newer feathers should have lower metal concentrations than older feathers. If nestlings are used, little variation due to growth sequence can be expected; however, as mentioned above, part-grown feathers can over- or under-estimate the body burdens of certain metals (Walsh, 1988; Burger & Gochfeld, 1992a), and results should therefore be assessed with this in mind.

Contaminants may be deposited exogenously onto the surfaces of feathers as well as sequestered endogenously from the blood into growing feathers, and thus the use of feathers as indicators of internal concentrations may be confounded by a combination of these two processes. Contamination from secretions of the uropygial gland smeared onto feathers during preening may also occur (Dauwe *et al.*, 2002b). In nestlings exogenous contamination onto the feather surface may be limited, and the growing feathers of nestling birds may thus represent the body load of contaminants better than those of adults (Dauwe *et al.*, 2004), although again it must also be borne in mind that these part-grown feathers are still connected to the blood supply and thus may not reflect the full extent of body-burden of contaminants.

Although feather concentrations of metals are reported in a number of studies and a number of experiments have been undertaken to investigate the potential use of feathers as a method of assessing the internal tissue concentrations of mercury in birds, relatively few published data are available reporting the relationship between feather and tissue concentrations for other metals. It has been demonstrated experimentally that mercury in feathers is strongly bonded and

concentrations are not affected by storage or vigorous treatments (Appelquist *et al.*, 1984). However, this may not be the case for other contaminants, including other metals, and the usefulness of feathers as tools for monitoring internal heavy metal contamination requires further investigation. This study aims to further investigate the potential use of feathers as a means of monitoring contamination of heavy metals and selenium (Chapter 6).

1.4 Nesting density and breeding success

The selection of a suitable nesting site that provides optimal conditions for the successful production and survival of young is of prime importance to individual and species survival. Breeding site selection has been shown to have a direct effect on reproductive success in a number of studies, with many different nest site characteristics monitored, including level of vegetation cover (Burger & Gochfeld, 1981; Saliva & Burger, 1989; Yorio *et al.*, 1995; Miyazaki, 1996; Bosch & Sol, 1998; Confer, 2003; García-Borboroglu & Yorio, 2004), vegetation type (Burger, 1976; Mermoz & Reboreda, 1998; García-Borboroglu & Yorio, 2004), substrate type (Burger & Gochfeld, 1981; Burger & Gochfeld, 1987) and topography (Nettleship, 1972; Burger & Gochfeld, 1981; Sanz, 1998; García-Borboroglu & Yorio, 2004). The physical and biotic features of an area will affect the degree of exposure to the elements and to predators and pathogens (Partridge, 1978; Best & Stauffer, 1980; Cody, 1985; Martin & Roper, 1988).

Seabirds typically nest at high density in large colonies, and this close proximity of nesting birds to each other has resulted in adaptations which allow successful breeding at high density, such as changes in behaviour towards other individuals (Coulson *et al.*, 1982). Most models of habitat selection assume that the value of a habitat to an organism is negatively correlated with the density of individuals of the same species (conspecifics) in that area (Morris, 1989; Rosenzweig, 1991), because of increased intraspecific interference, local competition, parasite load, disease, and many other factors (Rosenzweig, 1991; Krause & Ruxton, 2002). However, colonial organisms such as seabirds choose to live at high densities and several recognised advantages of this high-density colonial lifestyle have been suggested as outweighing the costs (Stokes & Boersma, 2000). Such advantages include the use of limited high-quality habitat (Lack, 1967; Shields *et al.*, 1988), optimal location (Horn, 1968), foraging success (Ward & Zahavi, 1973) and predation avoidance (Kruuk, 1964; Siegel-Causey & Kharitonov, 1990; Krause & Ruxton, 2002). The documented positive effects on breeding success in colonial seabirds gave rise to the concept of 'social stimulation', where it is claimed that increased

colony size gives rise to more efficient breeding, rather than the depressive effects associated with high density in many organisms (Darling, 1938).

In colonial seabirds, individuals breeding in certain areas of the colony have shown marked differences in breeding success compared to those in other areas (Nettleship, 1972; Hudson, 1982; Pierotti, 1982; Bosch & Sol, 1998), and individuals placing nests in optimal microhabitats are more successful in fledging young than those occupying less favourable locations (Gochfeld, 1978; Yahner, 1983). However, competition for the best breeding sites may result in a positive association between individual quality and habitat quality, with higher quality birds tending to secure better habitat (Kim & Monaghan, 2005). Various studies have shown a positive relation between breeding performance and parental quality, as reflected in egg size, age and breeding experience (Ollason & Dunnet, 1978; Pugesek, 1981; Coulson & Porter, 1985; Bolton, 1991; Sydeman *et al.*, 1991; Ratcliffe *et al.*, 1998; Daunt *et al.*, 1999; Risch & Rohwer, 2000). Thus, when looking at breeding success, habitat quality and individual quality are often confounded and it is difficult to separate the effects of individual quality on breeding performance from effects that are attributable to physical components of the breeding areas (Kim & Monaghan, 2005). Although the effects of nest location and nesting density on reproductive success have been examined to some extent, results are conflicting and the relationship warrants further study. The effects of commercial egg harvesting on nest density have not previously been examined.

1.5 Black-headed gulls (*Larus ridibundus* L.)

The black-headed gull (*Larus ridibundus* L.) is the most common breeding gull in the British Isles and the most widely distributed breeding seabird in Britain and Ireland (Mitchell *et al.*, 2004). In Europe, many colonies occur along the north-western coasts as well as a few areas around the Mediterranean. The largest coastal colonies are found in Sweden, Denmark, The Netherlands and Britain (Cramp, 1983); in Britain, coastal colonies are largest in South and Southeast England and along the Irish Sea (Lloyd *et al.*, 1991). Black-headed Gulls also have an extensive inland breeding distribution, occurring in most European countries. In the UK, numbers breeding inland are similar to those on the coast, and the majority of the breeding population are resident throughout the year, with numbers increasing in the winter months with birds migrating from northern and eastern Europe, especially in the east and southeast of England (Mitchell *et al.*, 2004). Some migration does occur west to Ireland and south to North

and West Africa, although this is minimal compared to the numbers that remain resident in the UK (Malling Olsen & Larsson, 2004).

Like most gulls, black-headed gulls are omnivorous and feed on a wide variety of animal and vegetable matter. Feeding methods and diet vary considerably with location, season, food availability and individual (Cramp, 1983). Natural food sources include animal material, particularly insects and earthworms (particularly *Lumbriculus* spp.), and plant material, but feeding is frequently supplemented by household and industrial waste, with birds often seen scavenging in parks, gardens and campsites, where they will scavenge for bread, fish scraps and so on, on refuse tips, waste grounds, and at sewage works and sewage outlets (Cramp, 1983). Fish from lakes and rivers are a particularly important food source in early spring and in the autumn and winter months when insects are less frequent, and ground bait left by fishermen on the edges of lakes, gravel pits or reservoirs will also be scavenged (Vernon, 1972). Black–headed gulls have been observed to take frogs and field-mice, and during the nesting season both eggs and young birds of several species are preyed upon. Both intra- and interspecific kleptoparasitism (i.e. theft of food from other individuals) is frequently practised. On salt marsh and mudflats, black-headed gulls feed on the surface fauna of estuarine mudflats exposed at low tide and in the shallow water in a narrow zone below the tide line, including polychaete worms, various molluscs and small crabs, shrimps, sandhoppers and shore fly larvae and adults (Vernon, 1972).

Black-headed gulls produce semi-altricial young (Cramp & Simmons, 1983), i.e. chicks hatching covered with down and with eyes open, but incapable of departing from the nest and are therefore entirely reliant on parental feeding (Nice, 1962). Nesting tends to be in dense colonies often of several thousands on open ground or in low vegetation, with habitats such as wetlands, bogs, marshes and artificial ponds favoured, (Malling Olsen & Larsson, 2004; Mitchell *et al.*, 2004). Salt marsh islands are popular nesting sites, although high tides may flood nests and birds then have to either relay or abandon breeding. However, salt marsh islands also provide the benefit of decreased predation, with relative freedom from mammalian predators (Tubbs, 1999).

In spite of being relatively common in the British Isles, black-headed gulls are on the 'Amber' list of the British Trust for Ornithology (BTO) Birds of Conservation Concern 2002-2007 (BTO, 2007), indicating that this species are of medium conservation concern. This BTO classification means that there has been a moderate (25-49%) decline in UK breeding populations over the last 25 years and that greater than or equal to 50% of the UK breeding population are located in ten

or fewer sites. Figure 1-3 provides a comparison of black-headed gull breeding occurrence in 10 km OS squares in Britain and Ireland during the Seabird 2000 census (1998-2002; Mitchell *et al.*, 2004) and the New Atlas of Breeding Birds (1989-1991; Gibbons *et al.*, 1993).

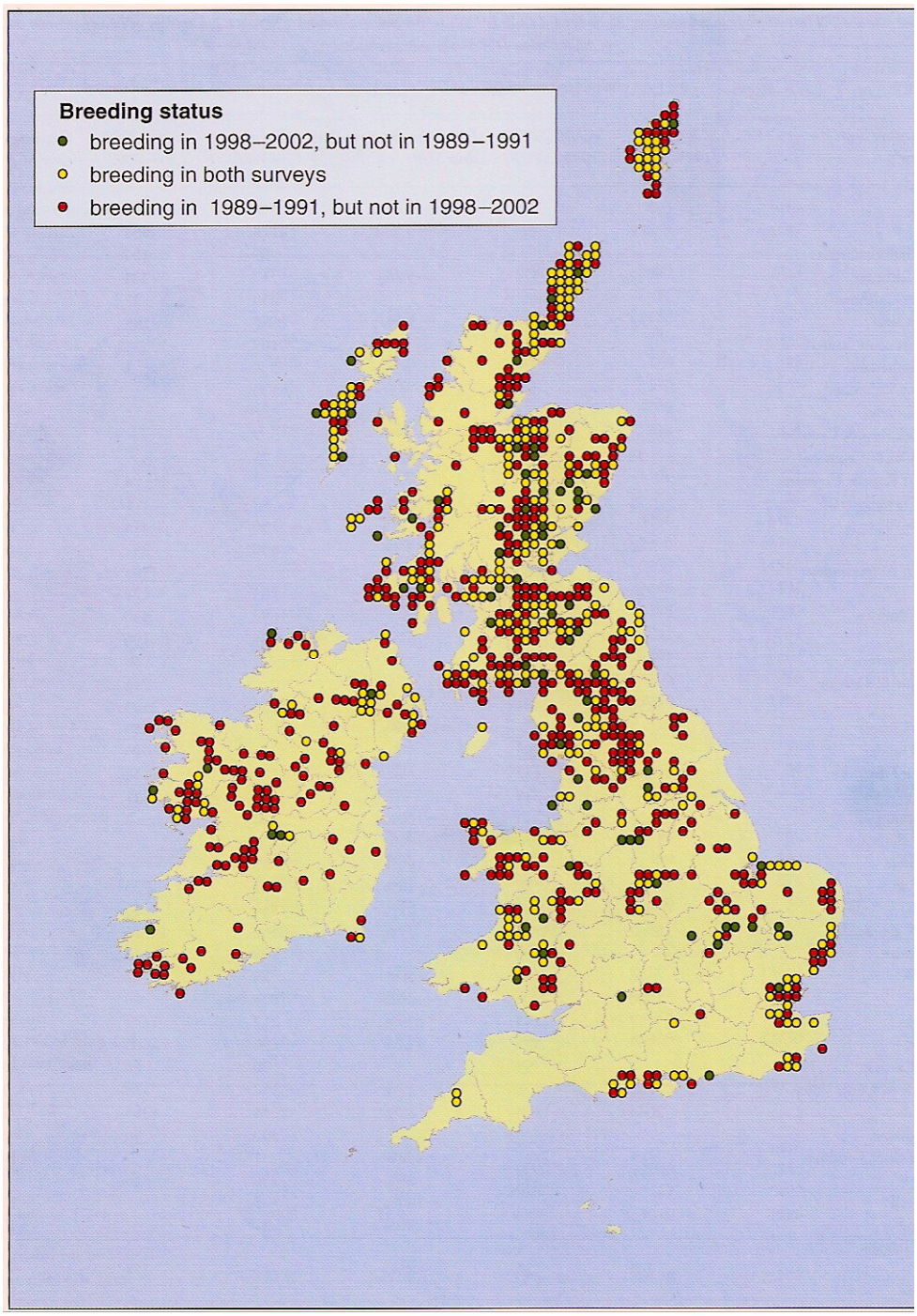


Figure 1-3 Black headed gull breeding status 1998-2002 and 1989-1991 (Mitchell *et al.*, 2004)

As can be seen in Figure 1-3, there are some populations that are new in the 1998-2002 census and were not present in 1989-1991 census (represented by the green dots). However, these new populations are far outweighed by the number of breeding populations that have disappeared since the 1989-1991 survey (represented by the red dots), indicating an overall decline in the UK breeding populations of black-headed gulls over approximately a 10 year period.

Although there are relatively few sites supporting black-headed gull colonies, the colonies generally support large numbers of birds, making them easy to sample and also meaning that a license to enter the colonies for sampling purposes may be obtained, which may not be possible for many other species of conservation concern. Black-headed gulls can also be used as an indicator species to provide information on the degree of pollution in other, rarer seabird species, for example the Mediterranean gull (*Larus melanocephalus*), a rare species in the UK which breeds in small numbers at the Lymington and Poole sites.

1.5.1 Egg laying and commercial egg collecting

Black-headed gull eggs are collected at a number of sites across the UK, for personal and commercial consumption. Since the Wildlife and Countryside Act 1981, it has been necessary to obtain a license to collect black-headed gull eggs. Licensing is through the UK Department of the Environment, Food and Rural Affairs (Defra), in collaboration with Natural England. The marsh sites between Lymington and Hurst Spit are harvested for black-headed gull eggs for commercial purposes under license. The license allows collection from the start of the laying period until the 15th May, and all eggs are collected daily from the harvested site. Egg-collecting begins at the very start of the breeding season, and the gulls continue to lay new eggs every time they are collected. By the end of the collecting period females have been laying continuously for several weeks. The Raby colony is also collected under Defra/Natural England license, but collection is undertaken by the estate manager at a very low level, for non-commercial purposes.

A typical clutch of black-headed gull eggs contains three eggs and, like most Laridae, if eggs are lost within a few hours of the first being laid, the gulls will add another egg to the same clutch, or if the entire clutch is lost, the whole clutch may be replaced. The former is known as ‘protracted’ laying, the latter as ‘replacement’ laying. Black-headed gulls will stop protracted laying after losing up to seven eggs consecutively, and replacement of entire clutches is also dependant on the time of season and the number of clutches previously laid (Weidmann, 1956). Although gulls will relay quite late in the breeding season, the 8-13 days of follicle growth

needed to produce a clutch requires high energy reserves, and forcing females to lay replacement eggs means that late breeders delayed by commercial collection must incur the costs of laying and incubating those extra eggs (Verhulst & Timbergen, 1991). The phenomenon of replacement laying is an adaptation to unpredictable factors such as nest wash-out due to flood, and predation (Brown & Morris, 1996). Studies of egg harvesting have reported reduced breeding success in some seabird populations at concentrations considered to pose a potential threat to viability (Feare, 1976a; Haynes, 1987; Vermeer *et al.*, 1991; Burger & Gochfeld, 1994; González, 1999; Zador *et al.*, 2005; Wood *et al.*, 2009) or to cause decline (Ainley & Lewis, 1974; de Juana, 1984; Shannon & Crawford, 1999); although some gull populations have also been successfully managed with egg harvesting (Wanless *et al.*, 1996; Ickes *et al.*, 1998).

Egg size has been reported to be reduced in replacement clutches (Feare, 1976b; Parsons, 1976a; Brown & Morris, 1996; Nager *et al.*, 2000; Hipfner *et al.*, 2003) and replacement clutches may contain fewer eggs (Brown & Morris, 1996) and show more abnormalities such as white or half-white eggs, small eggs, or eggs that took on a 'corroded', dried-out appearance, characterised by a cracked and partially collapsed shell and apparently thickened membrane (Wood, 2007). Increased egg production has been shown to reduce endogenous proteins in breeding females (Bolton *et al.*, 1993; Cooke *et al.*, 1995), which are used to form eggs and limit the production of replacement clutches and reduce egg size and egg quality, measured by yolk:albumen ratio and protein content (Robbins, 1981; Houston *et al.*, 1983; Bolton *et al.*, 1992; Monaghan *et al.*, 1998; Hipfner *et al.*, 2003; Wood *et al.*, 2009). Most of these endogenous proteins come from pectoral muscles (Houston *et al.*, 1995), depletion of which reduces flight performance and, as a result, reduces foraging efficiency and predator avoidance (Veasey *et al.*, 2000; Veasey *et al.*, 2001; Kullberg *et al.*, 2005). Muscle recovery during incubation is slow (Houston *et al.*, 1983) and thus the trade-off birds have to make between foraging to maintain their own body condition, and incubation and providing for the chick, is influenced (Monaghan *et al.*, 1998). The capacity for egg laying has been shown to be influenced by food supply (Oro *et al.*, 1999), so relaying may be more common in females in better feeding condition (Houston *et al.*, 1983; McNamara & Houston, 1996).

As a result of egg harvesting, the appearance of hatchlings on the Lymington colony is delayed by approximately three weeks. A number of authors report significantly reduced breeding success with late-laying birds, as opposed to early or peak layers (Patterson, 1965; Parsons, 1975; Becker, 1995). One explanation offered for this phenomenon is that synchronised breeding has an anti-predator function (Kruuk, 1964) and minimises the age-difference between

chicks on a colony, which in turn reduces the amount of food-robbing by larger chicks from their smaller counterparts (Perrins, 1970). On a collected colony breeding would still be synchronised as collecting ceases completely at a set time, and thus hatching would also be synchronised. However, the delay in hatching as a result of egg harvesting may have a detrimental effect on the reproductive success of birds in this colony for other reasons such as changes in weather conditions, temperature, and so on. For example, high spring tides occur annually at the start of the breeding season (during the second week in April) and again in mid- to late May (Wood, 2007), and these high waters can flood the marsh and wash-out nests, resulting in loss of chicks and eggs. The delay in hatching means that, in late May, eggs and young chicks are present which may be lost in these floods, from which older chicks would be better equipped to escape (Aspinall *et al.*, 1993).

Concentrations of pollutants have also been shown to vary through the clutch sequence, with the general observation being that concentrations are higher in the last laid egg than in the first laid (Becker, 1989). It has been suggested that this phenomenon occurs because birds produce their first eggs mainly from recent dietary uptake, whereas body reserves contribute more to later eggs (Mineau, 1982). If female birds lose a substantial portion of their body burden of pollutants during egg laying, concentrations in internal tissues might drop as they are sequestered in the eggs (Van den Steen *et al.*, 2006). Relaying a new clutch requires mobilising body reserves and increased feeding, both of which may lead to increased blood concentration of lipid-soluble pollutants (Helberg *et al.*, 2005). This last point may be particularly relevant in the case of the heavily collected Lymington colony, where females have laid continuously until collecting ceases and the last clutch is finally allowed to incubate and ultimately hatch.

The possible effect of laying order on the concentrations of contaminants in eggs within a clutch has been examined by several authors, and studies have shown that, with certain pollutants, there is a pattern of increasing concentration within the clutch related to the order of egg laying, with the third (usually final) egg containing the greatest concentration of pollutants. For example, concentrations of DDT have been reported as lowest in the first-laid egg compared with subsequently laid eggs within clutches of common terns (*Sterna hirundo*; Nisbet, 1982), herring gulls (*Larus argentatus*; Mineau, 1982) and great crested grebes (*Podiceps cristatus*; Lukowski, 1978). If birds are constantly relaying it is possible that the concentration of pollutants will be increased with each newly laid egg, and for birds on collected colonies, the difference between the contaminant load of first laid eggs at the true start of breeding and that of the first laid eggs after collection (i.e. the egg that will actually be allowed to develop and hatch) could be significant and may have implications for the fitness of the clutch. However, it is

important to note that, for metals, no clear relationship between laying order and egg concentrations of pollutants is reported (Becker *et al.*, 1989; Dauwe *et al.*, 2005), and for mercury a decrease in concentration through the laying sequence has been documented (Becker, 1992). Any relationship between contaminant concentration and laying order is far from clear, and concentration of metals has been examined in very few studies. This area therefore warrants further investigation.

1.6 Study areas

The sites sampled in this study are the Pylewell marshes at Lymington (Hampshire) and marshes at Poole Harbour (Dorset), both on the South coast of England. Eggs were also obtained from a small inland black-headed gull colony in the North of England (Raby Estate, North Pennines). A map of England, Scotland and Wales showing site locations is provided in Figure 1-4 and the site characteristics are summarised in Table 1-3. More detailed site maps are provided in Figures 1-5, 1-6 and 1-7. Detailed information regarding the potential sources of pollution at each of the sites is provided in Chapter 4.

Figure 1-4 Map of England, Scotland and Wales showing location of sampling sites

Table 1-3 Characteristics of Lymington, Poole and Raby sites

Site	Location	Grid reference	Colony size	Approx. no. breeding pairs	Colony type	Height	Average annual temperature*	Collection regime
Lymington (Pylewell colony)	Hampshire, South coast of England	SZ345942	Large	4300	Salt marsh island	Sea-level	10.6 - 11.6°C	High-level, commercial collection
Poole	Dorset, South coast of England	SY964902	Large	10000	Salt marsh island	Sea-level	10.6 - 11.6°C	Uncollected
Raby	County Durham, North East England	NY822291	Small	<500	Inland, nesting on wavy hair/cotton grass heather mix	*ca*. 500 m above sea-level	6.5 - 7.5°C	Low-level, non-commercial collection

* average annual temperature data from Met Office UK (Met Office UK, 2009).

Referring to the black-headed gull breeding status represented in Figure 1-3, the sites examined in this study support black-headed gull populations that were present in both the 1998-2002 census and the 1989-1991 census (represented by the yellow dots). However, a number of black-headed gull colonies were present in the 1989-1991 census in the area around all three sites examined in this study, that disappeared by the time of the 1998-2002 census (represented by the red dots); thus there has been an overall decline in the breeding populations of black-headed gulls in these areas.

The sites were chosen to provide comparisons between colonies exposed to different sources of pollution and different pressures to the colonies themselves. Whilst the Lymington and Poole colonies are both located on the South coast of England and both support relatively large populations, the two sites are impacted by different potential sources of pollution and, most notably, the Lymington colony is subject to commercial egg collecting while the Poole colony is undisturbed. The fact that the Lymington site is commercially collected adds an interesting extra dimension to the study, allowing comparisons to be made with pollutant loads and reproductive parameters such as egg size/quality between a collected and an uncollected colony. Collection of samples from the Lymington colony at both the start and the end of the egg collecting period will enable comparisons to be made not only with samples from the uncollected Poole colony, but also with differences between the first laid eggs at the start of the season and the first eggs in the final clutches laid after egg-collecting has ceased.

The Raby colony is very different to the Poole and Lymington South coast colonies. It is a small colony in the North of England and, being located inland, the birds will have very different feeding strategies to those nesting on the coast, and will thus be exposed to different sources and types of pollution. In addition, the colony is located on old lead mining dams,

which could provide a potential source of heavy metal exposure to the birds feeding and nesting in the area. Comparison of this colony with the South coast colonies will provide some insight into the differences between inland and coastal colonies and, owing to the very different climate in North East England compared to the South, may also provide information regarding any potential differences and adaptations of a black-headed gull colony to location in a cooler, upland climate. The Raby colony is subjected to a very low level of non-commercial collection.

1.6.1 Lymington

The Lymington marshes are located in the Lymington estuary, within the Solent (see Figure 1-5). The Solent is the drowned valley of a river which flowed between what are now the Isle of Wight and the mainland of Hampshire and West Sussex, on the south coast of England (Tubbs, 1999); it is one of the few major sheltered channel systems in European waters, and has a two-way flow separated by defined periods of slack water. The Solent experiences a 'double high water', i.e. there are two high tides in each cycle, which is a phenomenon unique to this area in Europe. Encompassing a major estuarine system, the Solent has the largest number of small estuaries in a tight cluster in Great Britain, and includes very important and complex marine and estuarine habitats, with unusual examples of natural transitions from marine to coastal and terrestrial habitats (English Nature, 1998). The deposition of sediment in the shallow, sheltered waters has resulted in the formation of mud and sand flats, and the marine sediment habitats are influenced by a range of salinities, exposures to wind and wave action and intensity of tidal streams. The higher parts of these mud and sand flats have been colonised by salt marsh vegetation. The extensive intertidal mudflats, salt marshes and shingle habitats within the Solent support nationally and internationally important numbers of nesting, roosting and feeding sea- and shorebirds, and the Solent constitutes one of the top five sites in the UK in terms of ornithological importance (Burges, 2000).

The marshes at Lymington are large salt marsh areas lying on opposite sides of the Lymington estuary. These sites are protected as part of the Solent Maritime Special Area of Conservation (SAC; under the EU Habitats Directive 92/43/EEC) and also lie within a Site of Special Scientific Interest (SSSI) and in the Solent and Southampton Water Ramsar Site (under the International Convention on Wetlands of International Importance) and Special Protection Area for birds (SPA; under the EU Birds Directive 79/409/EEC). These salt marshes are important breeding areas for black-headed gulls, and support a total of approximately 7600 breeding pairs, of which the specific colony sampled in this study - the salt marsh island at Pylewell - comprises

over 4300 pairs (Wood, 2007). The eggs of black-headed gulls are harvested from these sites, and all salt marshes between Lymington and Hurst Spit, for consumption and market, under license through the UK Department of the Environment Food and Rural Affairs (Defra) in collaboration with Natural England (see Section 1.5.1).

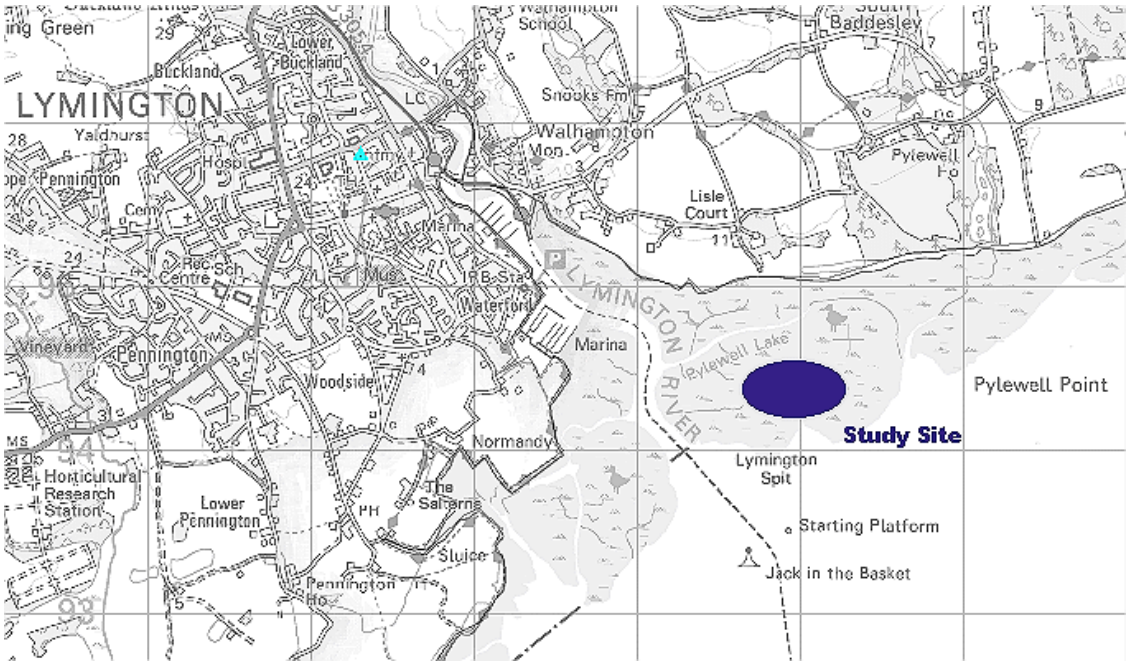


Figure 1-5 Map of Lymington showing sampling site (from www.magic.gov.uk)

1.6.2 Poole Harbour

Poole Harbour occupies a flooded shallow depression towards the south-western extremity of the Hampshire Basin (see Figure 1-6). The Harbour is a bar-built estuary (i.e. formed from the build-up of sandbars along the coastline); it is one of the largest natural harbours in the world and is of national and international importance for nature conservation. The narrow opening at Poole Bay means that a significant body of water may be retained throughout the tidal cycle, resulting in relatively low tidal energy in the Harbour and lagoon-type characteristics. The Harbour is one of 37 natural saline lagoons in England and Wales, and occupies three-quarters of the total area of 3300 hectares represented by this habitat (Langston *et al.*, 2003). As in the Solent, Poole Harbour experiences a double high water.

The range of estuarine, wetland and heathland habitats and the animals and plants they support, together with the large variety and number of birds, means that Poole Harbour is an area

recognised as being of international importance and holds a number of statutory designations which serve to protect the natural environment. Sites around the Harbour are designated as Areas of Outstanding Natural Beauty (AONBs) and the southern shores have Heritage Coast status. Parts of Poole Harbour are designated as SSSI and a SPA, and the Harbour and surrounding areas are recognised under the International Convention on Wetlands of International Importance as a Ramsar site. There are three National Nature Reserves (NNRs) and three Local Nature Reserves (LNRs), as well as reserves managed by the Dorset Wildlife Trust and the Royal Society for the Protection of Birds (RSPB). The wetland habitats bordering the Harbour support large numbers of wintering, migrating and breeding birds along with many rare plants and invertebrates. In particular, the mudflats and salt marshes are of great ecological value for feeding and roosting birds.

The main population of black-headed gulls in Poole Harbour is situated in colonies on three islands: a larger island bordered closely on either side by two smaller islands of approximately half the size. These salt marshes support a total of approximately 10000 breeding pairs of black-headed gulls. Although black-headed gull eggs were commercially harvested historically, this practise was prohibited in the early 1990's and since then eggs from the Poole Harbour colonies have not been harvested.

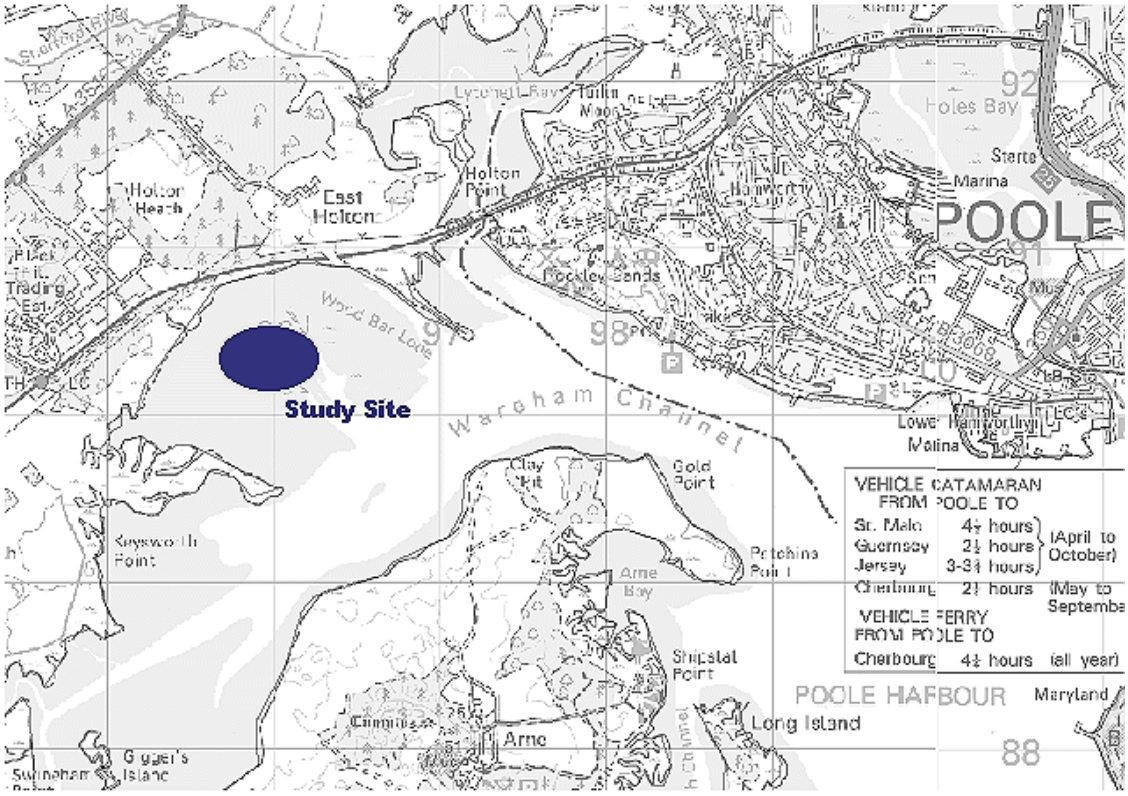


Figure 1-6 Map of Poole Harbour showing Poole sampling site (from www.magic.gov.uk)

1.6.3 Raby Estate

The Raby Estate is located in the North Pennines, which is classified as an AONB. A population of less than 500 pairs of black-headed gulls nest around a collection of small water bodies within the Estate, to the east of Cow Green Reservoir, south of Widdybank Fell and north of the River Tees, approximately 500 m above sea-level (Visit Cumbria, 2008). The land here is primarily blanket bog, typified by heather, wavy hair/cotton grass and bog moss, with some flushed areas dominated by rushes and sedges. The area around Widdybank Fell is of international importance for its acid grassland, moorland and blanket bog habitats, which support a variety of upland birds and, most notably, breeding waders. The site is also important for its rare arctic alpine plants, which are unique to Great Britain. As a result, this area is a NNR (the Moor House-Upper Teesdale NNR) and a SSSI, as well as being designated a SAC and a SPA. The Moor House-Upper Teesdale NNR is one of the largest (*ca*. 7400 ha) and most remote nature reserves in England, with some of the areas of the Raby Estate having restricted access due to the sensitive habitats they support.

The climate in the region is very different to the climate of the south coast sites. The moorland where the black-headed gulls nest lies at an altitude of around 500 m above sea level, and the region is strongly influenced by the prevailing Atlantic climate with cool summers and mild winters, and experiences high average wind speeds (24 km/hour), low average annual temperatures (*ca*. 5ºC; average monthly temperature minimum 0.1ºC in February, maximum 12.3ºC in July) and high average rainfall (1800mm; Turner *et al.*, 2003; Armitage, 2006). A map of the area and sample site is provided in Figure 1-7.

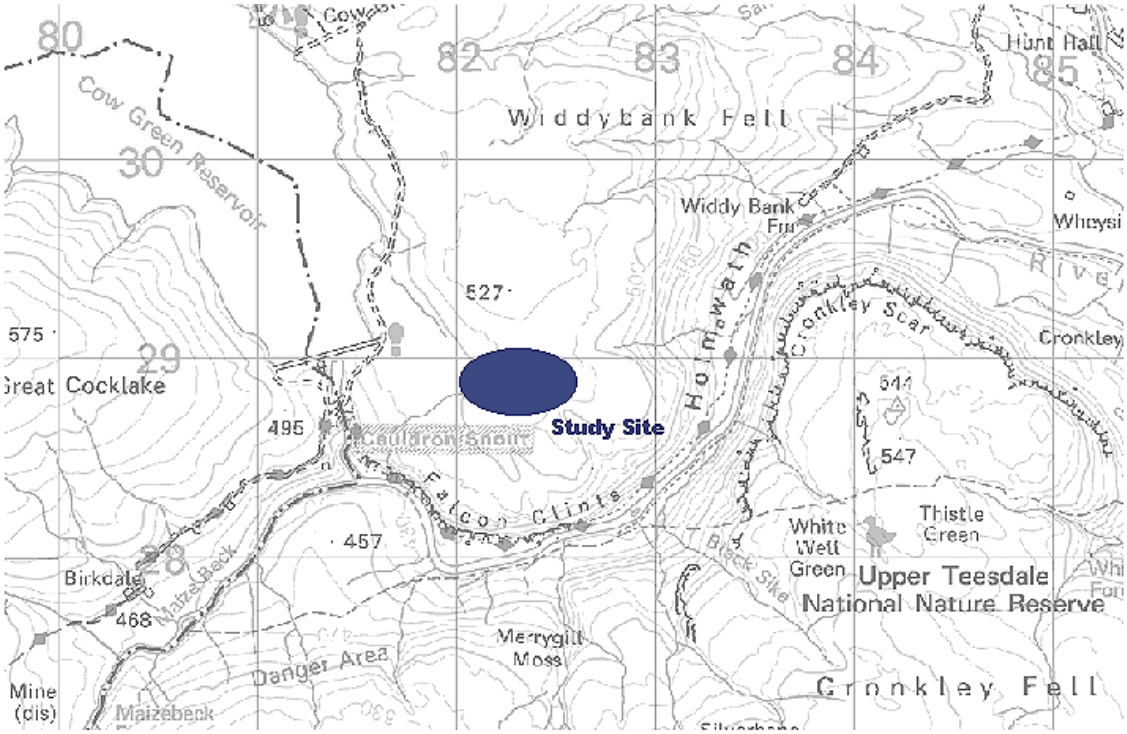


Figure 1-7 Map of Widdybank Fell area showing Raby Estate sample site (from www.magic.gov.uk)

The Raby Estate black-headed gull colony is collected at a very low level on behalf of the Estate Manager, for personal consumption. The eggs are not sold commercially and a very small number - between 18 and 40 annually - are taken (Waddell, *pers. comm.*). This practise is licensed through the UK Department of the Environment Food and Rural Affairs (Defra) in collaboration with Natural England.

1.7 Project aims and objectives

1.7.1 Aims

The principal aim of this project is to assess the concentrations of heavy metals and selenium in populations of black-headed gulls (*L. ridibundus*) from different breeding colonies in the UK which have different general characteristics and are subject to different sources, types and degrees of pollution, and to assess the suitability of eggs and feathers to provide an indication of local pollution.

The following questions will be addressed:

- What are the concentrations of heavy metals and the related semi-metal selenium in the eggs and feathers of black-headed gulls at the sites investigated?
- How are the metals partitioned between the different egg components (yolk, albumen and shell)?
- Do the concentrations of the metals investigated have any significant impact on the shell thickness, composition and quality of black-headed gull eggs?
- Does commercial collection of black-headed gull eggs have an impact on the concentration of the metals investigated in eggs?
- Are feathers a useful tool for non-destructive monitoring of heavy metals and selenium?
- What differences can be observed between the degree and specific type of pollution between the sites investigated in this study?
- Does nesting density affect the egg size of the black-headed gull, and could it be a confounding factor for other elements of study?

1.7.2 Objectives

In order to achieve the aims and answer the questions outlined above, the following research objectives have been identified:

1) Take measurements of eggshell thickness, egg size and the weights of egg components (i.e. yolk, albumen and shell) from three colonies: an uncollected colony on the South

coast of England (Poole), a colony on the South coast of England from which black-headed gull eggs are collected for commercial purposes (Lymington), and an inland colony in the North of England (Raby Estate), which is collected at a very low level and for non-commercial purposes. Test the following null hypotheses: *commercial egg collecting has no significant effect on the quality of black-headed gull eggs*; and *there are no significant differences between the physical characteristics of black-headed gull eggs from colonies located on the South coast of England and an inland colony in the North of England.*

2) Examine the concentrations of heavy metals and selenium in black-headed gull eggs (both total egg concentration and concentrations in yolk, albumen and shell) from the three colonies outlined in Objective 1. Test the following null hypothesis: *commercial egg collecting has no significant effect on the concentrations of heavy metals and selenium in black-headed gull eggs.*

3) Assess relationships between egg measurements from Objective 1 and metal concentrations in eggs from Objective 2 to investigate any trends or significant relationships. Test the following null hypothesis: *there are no significant interactions between concentrations of heavy metals and selenium measured in black-headed gull eggs and the physical characteristics and quality of the eggs.*

4) Use the results from Objective 2 to provide an indication of the extent of pollution at each of the sites and to make comparisons between sites according to the potential pollution sources in the surrounding area. Test the following null hypothesis: *concentrations of heavy metals and selenium in black-headed gull eggs do not provide an indication of local pollution.*

5) Relate the information on egg size, egg quality and concentrations of heavy metals and selenium in eggs gained from Objectives 1 and 2 to ecological outcomes, particularly effects on reproduction. Use the knowledge gained to examine the possible effects of commercial egg collecting on the reproductive success of black-headed gulls. Test the following null hypothesis: *commercial egg collecting has no significant effect on the breeding success of black-headed gulls.*

6) Investigate the variation in egg sizes in nests from different areas of salt marsh island colonies (areas densely populated with nests versus less densely nested areas). Use the data to make an assessment of which conditions are preferable for nesting for black-

headed gulls on salt marsh islands, and to provide information on the general fitness of the birds in the colony. In addition, the sampling strategy employed for egg collection may be validated by the results. Test the following null hypotheses: *nest density has no significant effect on egg size*; and *commercial egg collecting has no significant effect on nest density*.

7) Examine the concentrations of heavy metals and selenium in black-headed gull chick down from two colonies on the South coast of England, one subjected to commercial egg collection and one uncollected (Lymington and Poole). Examine the suitability of feathers as a tool for measuring heavy metal contamination. Test the following null hypotheses: *concentrations of heavy metals and selenium in black-headed gull chick down do not reflect concentrations in black-headed gull eggs and local pollution sources.*

1.8 Thesis outline

Chapter 2 provides details of the sampling strategy for the eggs examined in both Chapter 2 and Chapter 5, and investigates variations in the size, dimensions and composition of black-headed gull eggs between the sites examined in this study (Objective 1). The differences in egg size, dimensions and composition between a site on which black-headed gull eggs are harvested for commercial purposes (Lymington), an unharvested site (Poole) and a site subject to low-level non-commercial collection (Raby) are assessed, and comparisons are be made between eggs of black-headed gull colonies breeding on salt-marsh islands off the South coast of England (Poole and Lymington) and those from a colony nesting inland on blanket bog (Raby). The wet weight of the whole egg and of each of the components (yolk, albumen and shell), egg volume, yolk:albumen ratio, egg length and breadth, shell thickness and shell index are examined. Data from this chapter will be further examined in Chapter 5 in order to assess the relationship between metal concentrations in eggs and potential impacts on the breeding success of black-headed gulls (Objectives 3 and 5), as indicated by the physical attributes of the egg.

Chapter 3 examines the impacts of nesting density on the size of black-headed gull eggs nesting on two South coast colonies in England, one subject to commercial egg harvesting (Lymington), and one unharvested site (Poole). Two separate areas of the colony are examined: a central, densely nested area of the colony, and a less densely nested area towards the edge of the colony. Comparisons are made between the size of eggs (length, breadth and volume) from

each of these colony areas, and the relationship between nesting density and egg size is investigated (Objective 6). This chapter will also test the validity of the sampling strategy used, as described in Chapter 2.

Chapter 4 provides an assessment of the potential sources of pollution around each of the study sites, within the foraging radius of breeding black-headed gulls. Potential sources of pollution discussed include industry (including fuel and power, metal, mineral and chemical industries), waste (landfill, sewage and waste treatment), boats and shipping, local land use (including roads, agricultural land and urban areas). The information from this chapter will be used to link in with the metal concentrations in black-headed gull eggs in Chapter 5, in order to identify possible sources of exposure to metal pollution impacting on the colonies (Objective 4).

Chapter 5 assesses the concentrations of heavy metals and selenium in black-headed gull eggs. Differences in the concentrations of heavy metals and selenium in eggs from a commercially harvested site (Lymington), an unharvested site (Poole) on the South coast of England and an inland site in the North East of England subject to low-level, non-commercial harvesting (Raby) are compared (Objective 2). Data for egg size, dimensions and composition from Chapter 2 are assessed alongside the metals data, to examine the effect of heavy metals and selenium on physical attributes of the egg Objectives 3 and 5). Information for potential pollution sources around each of the sites, discussed in Chapter 4, is examined alongside the metals data, in order to identify potential sources of the metal concentrations reported in the eggs.

Chapter 6 assesses the concentrations of heavy metals and selenium in black-headed gull feathers, specifically chick down. Differences in the concentrations of heavy metals and selenium in feathers from chicks on a commercially harvested site (Lymington) and an unharvested site (Poole) are compared to assess potential impacts of commercial egg harvesting on metal concentrations in the down of chicks. The correlations between metal concentrations measured in chick down and concentrations measured in eggs is examined (Objective 7), and the effectiveness of feathers as a tool for non-destructive monitoring of metal pollution is discussed.

CHAPTER 2. HOW DO THE SIZE, DIMENSIONS AND COMPOSITION OF BLACK-HEADED GULL EGGS VARY BETWEEN SITES?

2.1 Introduction

Egg size and composition (weight/volume of components and yolk:albumen ratio) have been shown to have a significant effect on the reproductive success of birds, with larger eggs producing larger chicks with an increased chance of survival (Parsons, 1970; Smith, 1974; Parsons, 1975; Nisbet, 1978; Lundberg & Väisänen, 1979; Moss *et al.*, 1981; Birkhead & Nettleship, 1982; Furness, 1983; Bolton, 1991; Styrsky *et al.*, 1999) and eggs with large yolks producing larger hatchlings (Carey, 1996; Finkler *et al.*, 1998; see Section 2.1.1). Eggshell quality has also been demonstrated to have a significant effect on breeding success; thinner shells lead to increased eggshell breakage and hatching failure (Hickey & Anderson, 1968; Enderson & Berger, 1970; Ratcliffe, 1970; Newton, 1973; Newton *et al.*, 1978; Newton *et al.*, 1983), and shell thickness affects the regulation of water loss from the egg (Booth & Seymour, 1987; Davis & Ackerman, 1987; Eeva & Lehikoinen, 1995; Nybø *et al.*, 1997; Helander *et al.*, 2002). Both of these factors have an impact on the overall breeding success of birds (see Section 2.1.2). The size, dimensions and composition of eggs may therefore be used as an indicator of the reproductive success of birds, and this chapter aims to assess the differences between black-headed gull egg size, dimensions and composition and relate these to the differences between the sites in terms of the impacts of the collection regimes, notwithstanding the concentrations of heavy metals and selenium in the egg. Data for metal concentrations will be analysed in conjunction with the data for egg characteristics in this chapter in Chapter 5, in order to assess the impacts of metals on black-headed gull egg size and structure.

2.1.1 Egg size and composition

Egg size can be measured easily, non-destructively and causes less disturbance than taking measurements of hatchlings. Size of egg (measured as total egg mass or egg volume in most studies) can be used as a measure of reproductive fitness for a number of bird species, and has been shown to reflect the intrinsic quality of the laying female and hence provide an indication of the quality of the colony as a whole (Parsons, 1970; Davis, 1975; Lundberg & Väisänen,

1979; Houston *et al.*, 1983; Wood, 2007). Increased egg production, for example as a result of birds forced to relay following egg harvesting, has been shown to reduce the condition of the laying female and the ability to rear young (Heaney & Monaghan, 1995; Monaghan *et al.*, 1998). If the quality of the laying bird is poor, foraging efficiency may be compromised and this may go some way towards explaining the connection between quality of the laying female and the size of the eggs laid: laying smaller eggs might be advantageous to the bird's individual breeding success, with smaller chicks hatching from smaller eggs requiring less food and, where large chicks might starve, smaller ones with a slower growth rate might survive (Perrins, 1996). Equally, good quality, healthy birds may produce larger chicks from larger eggs, as they are healthy enough to forage and provide for their chicks effectively.

Many studies have shown that larger eggs tend to produce larger, heavier chicks at hatching and chicks which have a growth advantage early in the nestling period over those hatching from smaller eggs (Smith, 1974, black-capped chickadee (*Poecile atricapillus*); Parsons, 1975, herring gull (*L. argentatus*); Nisbet, 1978, tern species; Lundberg & Väisänen, 1979, black-headed gull; Moss *et al.*, 1981, red grouse (*Lagopus lagopus scotias*); Birkhead & Nettleship, 1982, thick-billed murre (*Uria lomvia*); Furness, 1983, great skua (*Catharacta skua*); Bolton, 1991, lesser black-backed gull (*Larus fuscus*); Styrsky *et al.*, 1999, house wren (*Troglodytes aedon*)). An increased chance of survival for chicks from larger eggs has also been demonstrated with the herring gull (*L. argentatus*; Parsons, 1970). Large eggs are generally thought to be more successful because they provide the embryo with more and higher quality nutrients (Parsons, 1970); however, large eggs also confer some physiological advantages and provide a thermoregulatory advantage (Rhymer, 1988; Wiebe & Bortolotti, 1995). As egg size increases, the surface area:volume ratio decreases, meaning that the larger eggs retain heat more effectively and lose proportionately less water through evaporation (Drent 1970) and at a relatively lower rate (Carey et *al.* 1983). If the parent has to be away from the nest, larger eggs will therefore cool more slowly than smaller ones (Perrins, 1996). Birkhead and Nettleship (1982) suggest that female seabirds that lay late owing to the loss of the first clutch may be more successful (in terms of fledging mass of chicks) if they minimise the delay in laying by producing a small egg earlier, rather than further delay laying in order to develop and produce a larger egg. This would suggest that birds that have been forced to relay, either following natural egg loss or egg loss due to commercial egg harvesting, may lay smaller eggs in order that the time taken to reproduce the clutch is reduced.

It is also suggested that egg size reflects the quantity of yolk reserves available to the chick (Bolton, 1991), with some authors reporting that larger eggs contain more yolk (Ricklefs *et al.*,

1978). As yolk is the food reserve for the developing embryo, large-yolked eggs that carry more lipid energy for the chick during the embryonic stage may result in larger hatchlings, both in terms of skeletal size and in terms of mass (Carey, 1996; Finkler *et al.*, 1998). In a review by Williams (1994), egg size was found to explain on average 66% of the variation in chick mass at hatching, but only 30% of the variation in chick body size. When effects of hatching body size were controlled for, chick mass remained significantly correlated with egg size, but the reverse is not true (Williams, 1994). This suggests that large eggs give rise to heavier chicks at hatching, rather than structurally larger chicks. It has been demonstrated by other authors that chicks from large-yolked eggs hatch with more residual yolk reserves, that is, yolk which is unused at hatching and is withdrawn into the yolk sac of the chick and used to provide energy after hatching (Perrins, 1996). This residual yolk is crucial for survival during the first few days of life (Parsons, 1970; Lundberg & Väisänen, 1979). Lundberg and Väisänen (1979) observed that, for black-headed gulls, chick mortality in the first week depended strongly upon egg size and on the weight of the newly hatched chicks. If yolk reserves are a key factor in chick survival, yolk mass would influence chick growth for only the first few days, after which parental feeding would compensate for any differences in egg size (O'Connor, 1975). However, these first few days of life are critical, and it is during this period where high mortality often occurs (Parsons, 1970).

In contrast to the theory that a larger egg means greater yolk reserves, studies have also shown that, particularly for seabirds (Williams, 1994), although larger eggs contain absolutely greater quantities of all constituents, they contain relatively less yolk and more albumen than their smaller counterparts in terms of yolk:albumen ratio (Romanoff & Romanoff, 1949; Parsons, 1976a; Nisbet, 1978; Ricklefs *et al.*, 1978; Finkler *et al.*, 1998; Lessells *et al.*, 2002). As albumen is composed almost entirely of protein and water, these latter studies suggest that the differences between large and small eggs lie mainly in the protein and/or water content, rather than the lipid, which is considered by most to be the better indicator of egg 'quality' (Bolton, 1991; Williams, 1994). This would suggest that the yolk:albumen ratio in the egg provides a more accurate reflection of egg quality than individual measurements of albumen and yolk without compensating for differences in egg size. In contrast, some authors suggest that it is the albumen that is the primary determinant of hatchling size and hatchling success, either due to being the primary source of water in the egg (Simkiss, 1980; Finkler *et al.*, 1998), or through being a major source of protein in the egg, which is important for embryonic development (Nisbet, 1978).

It is important to consider the fact that egg size is often a reflection of age and quality of the parents (Coulson & White, 1958; Davis, 1975; Ricklefs, 1984; Bolton, 1991; Sydeman & Emslie, 1992; Williams, 1994) and this may therefore be a confounding factor in studies that assume increased chick weight, size, survival and so on are entirely a product of the size of the egg from which they hatched. Parents of higher quality and with greater breeding experience may also have better chick-rearing abilities (Birkhead & Nettleship, 1982; Bolton, 1991), which could also be a confounding variable in the egg size/chick survival relationship. In spite of this, data in a study by Bolton (1991), examining chick survival in the lesser black-backed gull (*L. fuscus*) in relation to egg size and parental quality, indicate that the advantages of large egg size are real and can act independently of parental quality. A number of studies have attempted to separate the effects of parental quality and egg size by exchanging eggs between nests (meaning birds that laid large eggs raise chicks from small eggs, and vice versa; Schifferli, 1973; Parsons, 1975; Nisbet, 1978; Reid & Boersma, 1990; Amundsen *et al.*, 1996; Hipfner & Gaston, 1999). These studies suggest that egg size does indeed influence chick success, particularly in the early stages of development, irrespective of parental quality.

The size of the eggs in a clutch has been shown to vary with clutch sequence, with the first laid 'a' egg being the largest, followed by the second 'b' egg, and the third (usually final in gulls) 'c' egg being smallest (Parsons, 1972). In herring gulls (*L. argentatus*), the c-egg is an average of 11% smaller than the a-egg, and the b-egg rarely more than 2% smaller than the a-egg (Parsons, 1972). This phenomenon has been reported for other species, with differences reported between a- and c-eggs of 9.4% for the lesser black-backed gull (*L. fuscus*; Paludan, 1951), 7.0% for the laughing gull (*L. atricilla*; Preston & Preston, 1953), and 7.3% for the black-legged kittiwake (*Rissa tridactyla*; Coulson, 1963). For black-headed gulls, Wood *et al.* (2009) report the c-egg to be significantly smaller than the a- and b-eggs. In this study eggs were sampled from nests containing only one egg, assuming this to be the first egg laid (i.e. the 'a' egg) and therefore eliminating any confounding influence of the position of the egg in the sequence of the clutch sampled in the investigations into differences in egg size between sites.

2.1.2 Eggshell thickness and shell index

The impacts of eggshell thinning are widely documented and include increased eggshell breakage and hatching failure, which can lead to a dramatic reduction in reproductive success (Hickey & Anderson, 1968; Enderson & Berger, 1970; Ratcliffe, 1970; Newton, 1973; Newton *et al.*, 1978; Newton *et al.*, 1983). Aside from breaking and cracking more easily, thin shells are

associated with increased water vapour conductance and hence desiccation. The pores and underlying shell membranes of the egg form a resistance to gas exchange and therefore regulate water loss; the diffusion capacity of eggshells is a compromise between the need for sufficient exchange of respiratory gases and the need to minimise water loss (Nybø *et al.*, 1997). Eggshell thinning has been shown to result in increased water loss (Booth & Seymour, 1987; Helander *et al.*, 2002), which can result in excess desiccation of the egg and the embryo and have a strong negative effect on hatching success (Davis & Ackerman, 1987; Eeva & Lehikoinen, 1995; Nybø *et al.*, 1997). On the other hand, shells which are too thick could also be problematic as they may limit the exchange of respiratory gases, or cause problems during hatching (Perrins, 1996). However, no reports were found that documented problems associated with overly thick shells and, in a study with commercial breeders, at no time did the eggs appear too thick to hatch properly, with eggs with thicker-than-average shells causing no apparent problems (Bennett, 1992). Unfortunately, no data could be found providing an indication of an 'optimum' shell thickness for black-headed gulls.

Eggshell thickness may depend on the amount of calcium that the birds can acquire (Pierotti & Annett, 1990; Perrins, 1996). The female has to obtain calcium in large quantities for the production of eggshells during the laying process; female gulls mobilise up to 10% of their skeletal mass per day for egg formation (Houston *et al.*, 1983), and feeding on calcium-rich foods may facilitate more rapid recovery of the breeding period, improving their capacity to rear their brood (Monaghan *et al.*, 1998). Birds of inferior quality and health are likely to have a reduced capacity for foraging in general, and may therefore struggle to access sufficient calcium-rich foods (as well as foods rich in other nutrients); as a result, lower quality birds may lay eggs with lower quality, thinner shells.

Although direct measurements of shell thickness can be made using a micrometer, the surface area:volume ratio decreases as egg size increases, and it is therefore also beneficial to measure some kind of index of thickness based on the egg dimensions. The most commonly used is the Shell Index devised by Ratcliffe (also known as the Ratcliffe Index; Ratcliffe, 1967), which is described below in Section 2.2.2. By calculating an index of shell thickness based on shell mass, egg length and breadth a thickness based on the entire surface area of the shell can be obtained.

2.1.3 Impacts of egg harvesting on egg quality

As previously mentioned, reduction in egg size in replacement clutches has been reported by a number of authors, both for gulls (Parsons, 1976a; Brown & Morris, 1996; Nager *et al.*, 2000), and for other seabird species (Feare, 1976b; Hipfner *et al.*, 2003). Only one previous study could be found examining the effects of egg harvesting on egg quality: Wood *et al.* (2009), studied several black-headed gull colonies in Dorset and Hampshire, including the colonies examined in this study. The study found that the quality of eggs taken from the commercially harvested colonies was inferior to those from the unharvested colonies in several respects. Egg size in terms of volume was significantly greater and ratio of yolk:albumen was significantly higher in eggs from the uncollected colonies compared to the collected colonies. However, it should be noted that, in the case of the Wood *et al.* (2009) study, yolk:albumen ratios were calculated based on volume, whereas in this study the ratio is based on mass, which is the more widely used method of calculating yolk:albumen ratio (Scott & Warren, 1941; Ricklefs, 1977; Meathrel & Ryder, 1987; Hussein *et al.*, 1988; Harms & Hussein, 1993; Yannakopoulos *et al.*, 1998; Kuchida *et al.*, 1999; Kilpi *et al.*, 2008) and allows comparison with ratios reported in other studies. Eggshell thickness was also measured, and results showed that the eggs from the uncollected colonies had significantly thicker shells than those from the collected colonies. The study also examined the numbers of abnormal and failed eggs on each of the different colonies, and found that the collected colonies had a significantly higher proportion of failed eggs, and a significantly higher proportion of white eggs (i.e. shells without pigmentation), half-white eggs, abnormally small and yolkless eggs, and eggs with corroded shells (see also Section 1.5.1).

2.2 Methods

2.2.1 Egg collection: 2005 & 2006

Under appropriate English Nature (now Natural England) permits ('License to Kill, Take or Have in Possession Wild Birds' Eggs' and 'License to Disturb Schedule 1 Birds for Science, Research or Conservation'; License numbers 20051226 and 20061003) and permission from the land owners, black-headed gull eggs were collected at Lymington and Poole during April/May 2005 and 2006 (for site details, refer to Section 1.6). From the Lymington site, ten eggs were collected at the start of the breeding season, prior to the commencement of the commercial egg harvesting period in 2005 and 2006; these eggs are referred to as 'Lymington Early' eggs. A further ten eggs were collected from the Lymington site from the first clutches laid after the

commercial egg harvesting period had ceased, also in 2005 and 2006; these are termed 'Lymington Late' eggs. In Poole ten eggs were collected from the breeding site during the peak of the laying period in both 2005 and 2006. In 2006 only, ten eggs were obtained from the Raby Estate in the North Pennines. Again, eggs were collected during the peak laying period. In the post-collection samples from the Lymington colony in 2005, one of the eggs was damaged during transit to such an extent that it was not fit for examination, and thus only nine eggs were examined in the 2005 'Lymington Late' sample set.

In accordance with recommendations in the *Seabird Monitoring Handbook* (Walsh *et al.*, 1995), each visit to a colony was kept to one hour or less in order to reduce chilling of chicks and eggs, and colonies were not entered during rainfall or strong cold winds. At each of the sites, eggs were sampled from nests containing only one egg, assuming these to be the first laid, in order to ensure that eggs were fresh and would not have started to develop. Where eggs were found to be cold they were assume to be abandoned/addled and were not sampled. Sampling of eggs from nests containing only one egg - likely to be the first laid - also meant that the eggs sampled were from birds at approximately the same laying stage. The majority of nests on the colonies at the time of sampling contained only one egg, and as such this was assumed to be the start of the peak laying period. Eggs were also sampled from nests located in the centre of the colony where nesting was most dense, assuming this to be the most desirable nesting area and thus secured by the birds in the best condition. This method was employed in order to ensure eggs of birds of similar 'typical' quality were sampled, as it has been demonstrated that birds nesting outside of the main colony areas are often of lower quality in terms of reproductive success (Patterson, 1965; Gochfeld, 1978; Yahner, 1983). Nest site selection, breeding bird quality and impacts on breeding success are discussed in detail in Chapter 3.

Eggs were stored frozen, intact, pending analysis. Freezing eggs as a whole unit minimises water loss and any potential loss of volatile contaminants from the egg contents (Mora *et al.*, 2008). All egg samples were treated in the same way in order to maintain uniformity in the study.

2.2.2 Egg measurements

The outer dimensions (maximum width and length) of each egg were measured using vernier callipers (accurate to ±0.1 mm) and the egg volume estimated using the following calculation

(from Hoyt, 1979):

$$V = K_v \cdot L \cdot B^2 \qquad \text{(Equation 2.1)}$$

Where V = volume, K_v = volume coefficient, L = length and B = maximum diameter.

Although species-specific volume coefficients have been developed for a number of species, Hoyt (1979) suggests that volume can be estimated with a common volume coefficient of 0.51 that is applicable to eggs of most species (with the exception of species in which eggs are very pointed); thus a value of 0.51 was used for K_v in calculating the volume of the eggs in this study. This method has been previously used for the measurement of black-headed gull eggs by Wood *et al.* (2009).

Shell index (SI; also known as Ratcliffe Index) was calculated as follows (from Ratcliffe, 1967):

$$SI = m/lb \qquad \text{(Equation 2.2)}$$

Where m = wet weight of the eggshell, l = egg length, and b = egg breadth.

This calculation for shell index has been used by a number of authors as a measure of eggshell thinning, (Peakall & Lincer, 1996; Nybø *et al.*, 1997; Pain *et al.*, 1999; Helander *et al.*, 2002) and enables comparison of the results from this study with a range of others. By calculating an index of shell thickness based on shell mass, egg length and breadth, a thickness based on the entire surface area of the shell is obtained. Eggshell thickness was also measured directly (see below), to enable comparisons with literature data where this measurement was made.

Prior to analysis, eggs were thawed, weighed whole to obtain a total egg mass and then carefully cut open with a scalpel. The egg contents were removed and separated, and the weights of the yolk and albumen recorded. Egg yolks were rolled on a clean sheet of lab roll, in order to remove any excess albumen. Eggshells were washed in Milli-Q® water and dried, before measurement of the eggshell thickness at four points around the equator of the egg, using an adapted spring closing micrometer (±0.01 mm) with a rounded tip at the point of contact to fit the curvature of the shell.

The variables/parameters measured/calculated were as follows: wet weight of the total egg, wet weight of albumen (direct measurement), wet weight of albumen (calculated from total egg weight less weight of yolk and shell), wet weight of yolk, wet weight of shell, yolk:albumen ratio, maximum egg length, maximum egg width, total egg volume, shell thickness and shell

index. All weights were measured in grams or milligrams and length, width and shell thicknesses measured in millimetres. Egg volume was calculated using the Hoyt (1979) equation (Equation 2.1) and shell index calculated using the Ratcliffe (1967) calculation (Equation 2.2), previously described. Some loss of albumen occurred during egg separation as, owing to its attachment to the yolk, it was not always possible to remove the albumen completely and the yolk needed to be gently rolled on a sheet of lab paper to remove the excess, which it was not possible to recover. Thus two measurements of wet albumen mass were recorded - one measured and one calculated from the subtraction of the wet weights of the yolk and shell from the wet weight of the whole egg, to obtain a calculated albumen wet weight. Owing to the loss of albumen during the separation process, the calculated mass of albumen is assumed to be the more accurate measure, and thus the yolk:albumen ratio was computed using the calculated wet weight of albumen, and the calculated mass of albumen was used in all further examination of the results.

2.3 Results

Figures 2-1 to 2-7 provide bar charts of the means for each of the sites (mean of combined 2005 and 2006 data for Lymington and Poole, mean of 2006 data for Raby). Note: 'Lym Early' and 'Lym Late' refer to Lymington Early and Lymington Late eggs, as described in Section 2.2.1. N = 69: n = 20, 19, 20 and 10 for Lymington Early, Lymington Late, Poole and Raby sample sets, respectively.

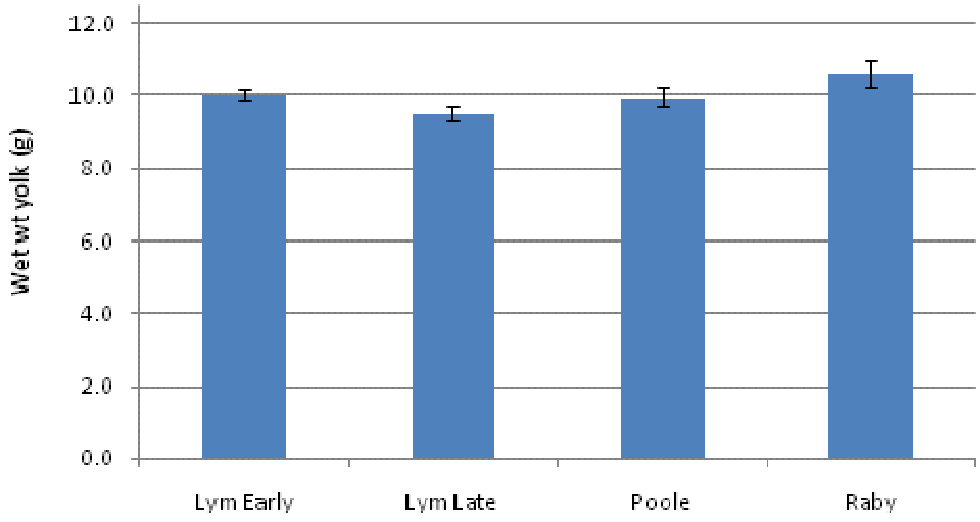


Figure 2-1 **Mean (± standard error) yolk wet weight for black-headed gull eggs from different sites, 2005-2006 (N = 69)**

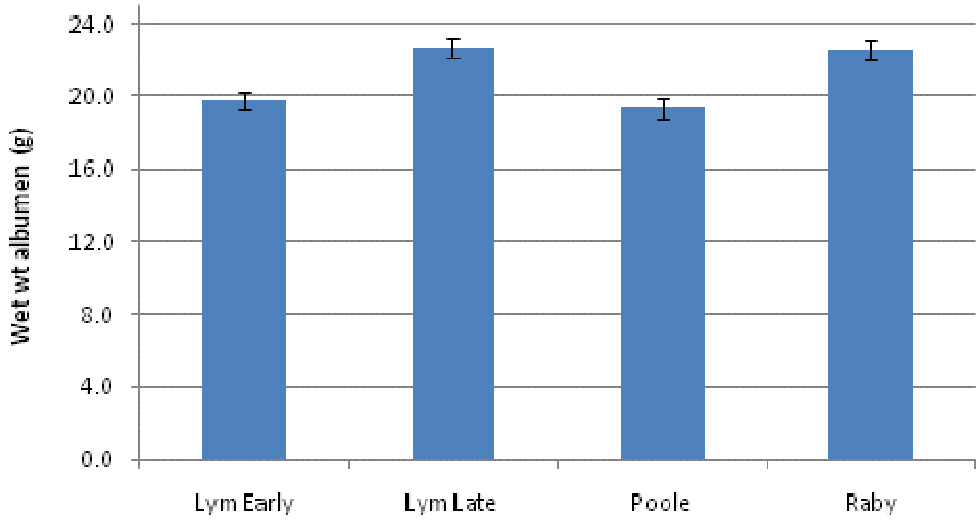


Figure 2-2 **Mean (± standard error) albumen wet weight for black-headed gull eggs from different sites, 2005-2006 (N = 69)**

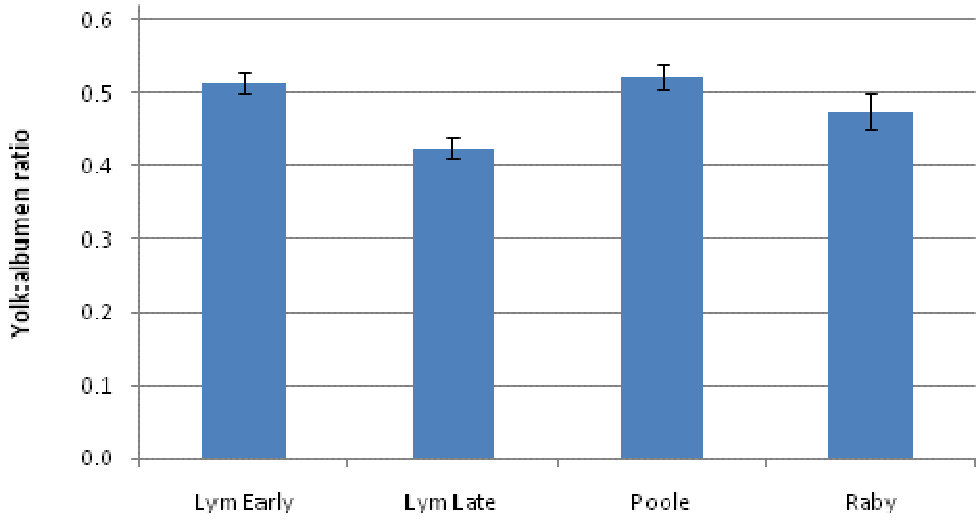


Figure 2-3 **Mean (± standard error) yolk:albumen ratio for black-headed gull eggs from different sites, 2005-2006 (N = 69)**

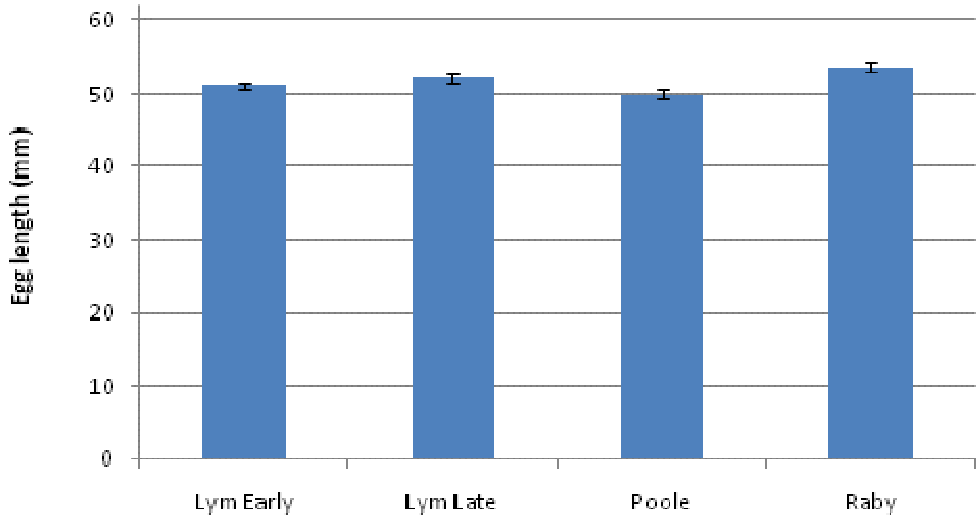


Figure 2-4 **Mean (± standard error) maximum egg length for black-headed gull eggs from different sites, 2005-2006 (N = 69)**

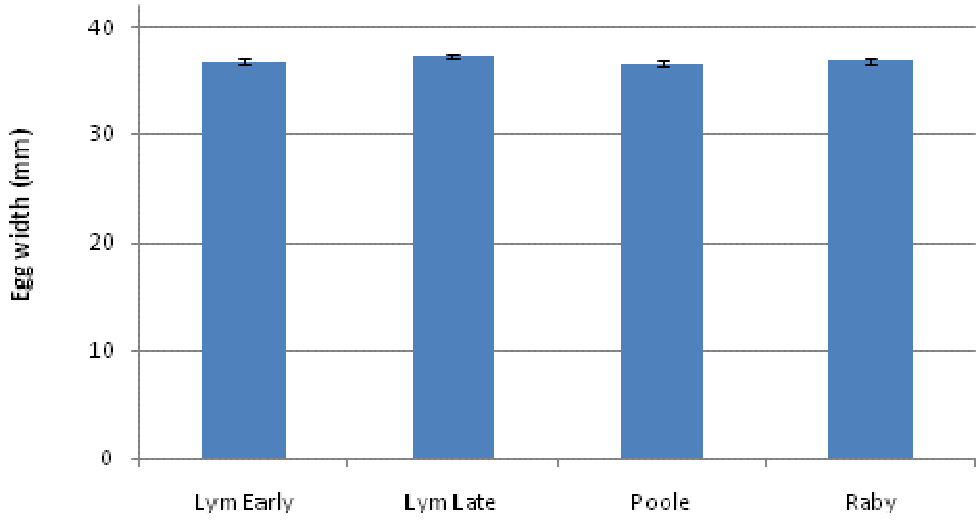


Figure 2-5 **Mean (± standard error) maximum egg width for black-headed gull eggs from different sites, 2005-2006 (N = 69)**

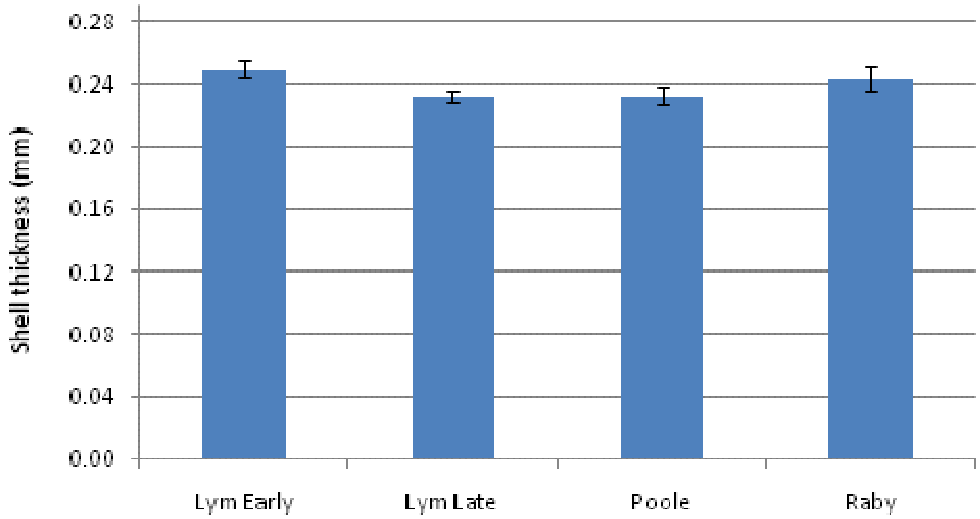


Figure 2-6 **Mean (± standard error) shell thickness for black-headed gull eggs from different sites, 2005-2006 (N = 69)**

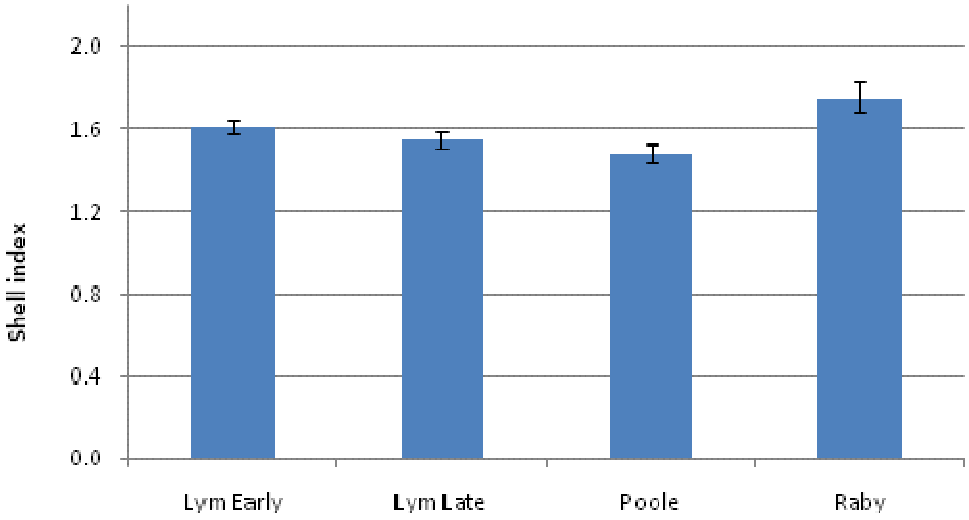


Figure 2-7 Mean (± standard error) shell index for black-headed gull eggs from different sites, 2005-2006 (N = 69)

Statistical analysis was carried out on the whole dataset, i.e. combined 2005 and 2006 data, for the Lymington and Poole samples. Data were pooled in this way to increase the sample sizes for analysis and to provide a general overview of the differences between the sites in terms of the physical characteristics of the eggs. In addition, no eggs were sampled at the Raby site in 2005 and egg data were therefore only for 2006 for this site.

In order to meet the requirements of parametric tests, data must be independent, normally distributed and have equal variance (Townend, 2002). In this dataset not all data were independent as in some cases a parameter was calculated using a measured variable; for example, the wet weight of albumen was calculated from the wet weight of the whole egg less the yolk and shell wet weights, the total egg volume was calculated using the length and breadth of the egg, and the shell index was calculated using the wet weight of the shell divided by the length and breadth of the egg. To maintain independence of the data, total wet weight of the egg, total egg volume and wet weight of the eggshell were not included in further statistical analysis. The total wet weight of the egg is reflected in the wet weights of the individual egg components and it was therefore not considered necessary for this measurement to be included in the subsequent statistical analysis. The total egg volume was removed from the dataset in preference to retaining the measured variables of egg length and breadth. Shell index was retained in the analysis in place of the wet weight of the shell, as the shell mass is included in

the shell index calculation, and shell index is considered to be the better measure of shell quality as it takes into account the thickness based on the entire surface area of the shell (see Section 2.2.2).

Normal distribution of the data was assessed using the Kolmogorov-Smirnov (K-S) test, which acts as a goodness-of-fit test, comparing the distribution in the data set being tested with the distribution of normally distributed data (Townend, 2002). If $p \leq 0.05$ the data are not normally distributed and may need to be transformed for the purposes of further statistical tests, or analysed using non-parametric tests. K-S test, which revealed that all data were normally distributed ($p > 0.05$), with the exception of the data for maximum egg width ($p = 0.02$). From the raw data there appears to be very little variation in the measurements of maximum egg width, and transforming the data (logarithmic, reciprocal, squared, cubed, square root, inverse square root, cube root and inverse cube root) did not result in a normal distribution. The raw data were therefore used in the subsequent statistical tests, but any significant difference in maximum egg width between sites should be treated with caution and subjected to further tests, owing to the lack of normal distribution.

Equal variance in the data can be examined using Levene's test of homogeneity of variance, which examines the variance across multiple samples (Field, 2005). Levene's test for homogeneity of variances revealed that all variances were equal ($p > 0.05$).

The differences between the sites were then investigated using a one-way analysis of variance (ANOVA) test to compare the means of the samples. ANOVA demonstrates that, for the variables/parameters where there are significant differences between the means for the different sites (populations), it can be concluded that at least two of the population means are significantly different. Table 2-1 provides a summary of the ANOVA data.

Table 2-1 ANOVA results for comparison of characteristics of black-headed gull eggs between sites, 2005-2006

	n	F value	p	Significance
Wet weight yolk				
Lym Early	20			
Lym Late	19	2.818	0.046	*
Poole	20			
Raby	10			
Wet weight albumen				
Lym Early	20			
Lym Late	19	10.14	<0.001	**
Poole	20			
Raby	10			
Yolk:Albumen ratio				
Lym Early	20			
Lym Late	19	7.724	<0.001	**
Poole	20			
Raby	10			
Egg length				
Lym Early	20			
Lym Late	19	5.214	0.003	**
Poole	20			
Raby	10			
Egg width				
Lym Early	20			
Lym Late	19	1.247	0.300	NS
Poole	20			
Raby	10			
Mean shell thickness				
Lym Early	20			
Lym Late	19	2.59	0.060	NS
Poole	20			
Raby	10			
Shell index				
Lym Early	20			
Lym Late	19	5.179	0.003	**
Poole	20			
Raby	10			

* = significant ($p \leq 0.05$); ** = highly significant ($p \leq 0.01$); NS = not significant.

ANOVA shows that there is a significant difference between the sites for wet weight of the yolk ($p = 0.046$), wet weight of the albumen ($p < 0.001$), the yolk:albumen ratio ($p < 0.001$), the maximum egg length ($p = 0.003$) and the shell index ($p = 0.003$). No significant differences between sample sites were found for maximum egg width or mean eggshell thickness.

Although ANOVA identifies significant differences between the different populations for each of the variables, it does not provide information as to exactly which populations are significantly different. In order to establish which of the sites differ for the variables, a post-hoc test is required. In this case, a Tukey test was used to compare all possible pairs of means and identify where the difference between two means is greater than the standard error would be expected to allow (Townend, 2002). The significant results from the Tukey test are presented in Table 2-2.

Table 2-2 Tukey test results for comparison of characteristics of black-headed gull eggs between sites, 2005-2006

	Significant site differences	n	Mean difference	S.E.	p	Significance
Wet weight yolk	Raby > Lym Late	19	1.09	0.380	0.028	*
Wet weight albumen	Lym Late > Lym Early	39	2.92	0.730	0.001	**
	Lym Late > Poole	39	3.28	0.730	<0.001	**
	Raby > Lym Early	30	2.77	0.882	0.013	*
	Raby > Poole	30	3.13	0.882	0.004	**
Y:A ratio	Lym Early > Lym Late	39	0.09	0.226	0.001	**
	Poole > Lym Late	39	0.1	0.226	<0.001	**
Max egg length	Raby > Poole	30	3.46	0.924	0.002	**
Max egg width	None	-	-	-	-	NS
Eggshell thickness	None	-	-	-	-	NS
Shell index	Raby > Lym Late	19	0.21	0.072	0.025	*
	Raby > Poole	30	0.27	0.071	0.002	**

* = significant ($p \leq 0.05$); ** = highly significant ($p \leq 0.01$); NS = not significant. S.E. = standard error.

The data from Figures 2-1 to 2-7 and the post-hoc Tukey test (Table 2-2) provide information as to which sites differ from one another and how. The results show that the 'Lymington Late' eggs - those taken from the Lymington colony after the commercial egg collecting period had

ceased - had a significantly greater mass of albumen than the Poole and the 'Lymington Early' (eggs taken at the start of the breeding season, as commercial egg collecting began) sample sets ($p = <0.001$ and 0.001, respectively). In terms of yolk:albumen ratio, however, the Poole and Lymington Early samples had a significantly greater yolk:albumen ratio than the Lymington Late eggs ($p = <0.001$ and 0.001, respectively). Eggs from the Raby site had a significantly greater yolk mass than those taken from Lymington after the collection period ($p = 0.028$), and were significantly longer than those from the Poole colony ($p = 0.002$). Although no significant differences were found between sites for eggshell thickness, the shell index for the Raby eggs was significantly higher than for both the Lymington Late and the Poole eggs ($p = 0.025$ and 0.002, respectively).

2.4 Discussion

Differences were observed between the Lymington eggs collected before the commercial egg harvesting period (Lymington Early) and those collected at the end of the commercial harvesting period (Lymington Late), i.e. those eggs that would have been allowed to develop and ultimately hatch. The Lymington Late eggs had a significantly greater mass of albumen than the Lymington Early eggs; however, in terms of yolk:albumen ratio, the Lymington Early eggs contained more yolk relative to egg size than the Lymington Late eggs. Egg size has been reported to be reduced in replacement clutches (Feare, 1976b; Parsons, 1976a; Brown & Morris, 1996; Nager *et al.*, 2000; Hipfner *et al.*, 2003) and increased egg production has been shown to reduce endogenous proteins (Bolton *et al.*, 1993; Cooke *et al.*, 1995), which are used to form eggs and limit the production of replacement clutches, reducing egg size and egg quality, measured by yolk:albumen ratio (Robbins, 1981; Houston *et al.*, 1983; Bolton *et al.*, 1992; Monaghan *et al.*, 1998; Hipfner *et al.*, 2003). The results for yolk:albumen ratio in this study indicate that the eggs of the Lymington colony laid prior to commercial egg collecting are of a greater intrinsic quality than those collected after the commercial egg harvesting period, as they contain greater relative yolk reserves for the developing embryo and newly-hatched chick.

The eggs from the uncollected Poole colony and the commercially collected Lymington colony exhibited some differences in terms of egg contents. Eggs from the Poole colony contained less albumen than those from the post-collection Lymington Late sample set, but in terms of yolk:albumen ratio the Poole eggs were significantly greater than the Lymington Late eggs. There was no statistically significant difference between the Poole and the Lymington Early eggs for either of these characteristics. Again, this would suggest that the eggs from the

uncollected Poole colony are of higher quality than those eggs sampled from Lymington at the end of the commercial collecting period.

In a previous study carried out on a number of colonies on the Dorset and Hampshire coast, including those colonies discussed in this study, the results also showed an adverse effect of commercial egg harvesting on eggshell thickness in black-headed gull eggs (Wood, 2007; Wood *et al.*, 2009), with eggs from collected colonies reported to have thinner shells than those from uncollected colonies. In the present study, although the eggs from the collected Lymington colony prior to the commercial collection period had thicker shells and a higher shell index than eggs from the same colony post-collection, this difference was not statistically significant. There were no significant differences between the Poole and Lymington colonies in terms of shell thickness or shell index. In fact, the pre-collection Lymington eggs had thicker shells and a higher mean shell index than the Poole eggs, although this was not statistically significant, and post-collection Lymington eggs had similar shell thicknesses, and a slightly higher (not statistically significant) mean shell index, compared to the Poole eggs. Therefore, the results of this study provide no indication that egg collection has a significant effect on eggshell quality.

Significant differences were observed between the small, inland Raby colony in North East England and the larger, coastal colonies on the South coast. Firstly, the Raby eggs were significantly longer than the eggs from the uncollected Poole colony. However, it should be noted that egg length has been shown to be a poor predictor of chick weight, and in fact chicks from broad short eggs have been found to be more successful than those from long narrow ones (Lundberg & Väisänen, 1979). Egg width is therefore considered to be more important than egg length in terms of breeding success, and no significant differences were observed between the Raby and Poole sites in terms of egg width in this study ($p = 0.30$), nor were any significant differences observed between eggs in term of egg width for any of the sites studied.

Eggs from the Raby site, which is subject to very low-level non-commercial harvesting, had a significantly greater mass of albumen than the pre-collection (Lymington Early) eggs and the Poole eggs, and a significantly greater wet weight of yolk than the eggs taken from the Lymington colony, post-collection (Lymington Late; Figure 2-1); no significant difference was found between the Raby colony and the pre-collection Lymington eggs (Lymington Early). The greater mass of yolk in the Raby eggs might also suggest that these eggs are higher quality than the Lymington Late eggs; however, no difference was observed between the two sites in terms of yolk:albumen ratio between eggs from the Raby site and any of South coast site eggs (Poole, Lymington Early, Lymington Late). The yolk:albumen ratio is thought to provide a more

accurate indication of egg quality than individual measurements of either yolk or albumen, as it takes into account the total egg mass (Bolton, 1991; Williams, 1994).

Although no significant differences were found between the shell thicknesses of the Raby eggs and the eggs from either Lymington (pre- or post-collection) or Poole, significant differences were found between eggshells in terms of shell index. Many authors consider shell index to be a more reliable measure of shell quality than simple measurements of shell thickness (Peakall & Lincer, 1996; Nybø *et al.*, 1997; Pain *et al.*, 1999; Helander *et al.*, 2002), as calculating an index of shell thickness based on shell mass, egg length and breadth, means that a thickness based on the entire surface area of the shell is obtained. The Raby eggs had a significantly higher average shell index than both the Poole eggs and the Lymington Late eggs ($p = 0.002$ and 0.025, respectively). No significant difference was found between the Raby eggs and the Lymington Early eggs.

As previously mentioned, eggshell thickness may depend on the amount of calcium that the birds can acquire (Pierotti & Annett, 1990; Perrins, 1996). Laying females that have been forced to relay several times owing to commercial harvesting of their eggs have been demonstrated to have reduced endogenous reserves and consequently reduced foraging efficiency (Monaghan & Nager, 1997). A reduced capacity for foraging may lead to difficulties in accessing sufficient calcium-rich foods (as well as foods rich in other nutrients) and, as a result, birds that have been forced to relay and are subsequently of a lower quality may lay eggs with lower quality, thinner shells. However, despite the relatively large mass of their eggshells, seabirds are not considered to be among those birds that selectively ingest calcium rich items before egg laying (Boersma *et al.*, 2004; Diller, 2004), most likely as a result of their varied diet consisting of a large proportion of invertebrates, fish and shellfish, which are foods rich in calcium. The results of the comparisons made in this study between two similar colonies in terms of size, type and location, but one commercially harvested and one unharvested (Lymington and Poole, respectively), suggest that commercial egg harvesting does not have a significant effect on shell thickness.

The eggshell has an extremely important role in the health of the developing embryo and must be protective and permeable to allow for successful embryonic growth and hatching. As a result, many authors suggest that eggshell thickness does not vary greatly within a species, rather the properties of the shell may be different in response to different environments (Rahn *et al.*, 1977; Carey, 1980; Visschedijka & Rahn, 1981). This may explain the fact the eggshell thickness/shell index was not found to vary significantly between the two colonies located on

the South coast of England, in spite of commercial collection on one of the sites, but the shell index was significantly higher for eggs from the North East Raby colony than those from the Poole and Lymington colonies (post-collection Lymington eggs significantly different, pre-collection Lymington eggs lower but not statistically significantly different). It may be that the black-headed gulls of the Raby colony have adapted according to the cooler climate of the North Pennines compared to the South coast of England, producing eggs with thicker shells to provide the eggs more protection against cooling too rapidly. The difference in shell index may also be attributable to a lower level of competition at the small Raby site than the large Poole colony (Raby colony comprises <500 pairs, Poole *ca.* 10000 pairs), or may be associated with the pollutant load of the eggs, which will be examined in Chapter 5.

The dimensions of black-headed gull eggs in this study appear to be very similar to those measured by other authors examining black-headed gull eggs. Table 2-3 shows measurements of length, breadth and volume for black-headed gull eggs from a number of studies (Rosenius, 1942; Ytreberg, 1956; Van Bree, 1957; Lundberg & Väisänen, 1979; Glutz von Blotzenheim & Bauer, 1982; Holz & Starke, 1984; Guthová, 1993; Svensson, 2002; Karlsson, 2005), in comparison with measurements made in this study. For uniformity in order to make comparisons, the egg volumes presented in this table were calculated from the length and breadth data provided by the authors, using the Hoyt (1979) method (Equation 2.1, Section 2.2.2) that is used throughout this study to calculate total egg volume.

Table 2-3 Comparison of egg length, breadth and volume in black-headed gull eggs from various sites worldwide

Site	N	Mean egg length (mm)	Mean egg breadth (mm)	Volume (cm^3)*	Reference
Sweden	205	52.48	37.29	37.22	Rosenius, 1942
Norway	624	51.40	36.31	34.56	Ytreberg, 1956
Texel, Netherlands	1246	51.50	36.72	35.41	Van Bree, 1957
Germany	1428	52.02	36.69	35.71	Glutz & Bauer, 1982
Croatia	1000	51.10	35.70	33.21	Glutz & Bauer, 1982
Germany	196	51.13	36.41	34.57	Holz & Starke, 1984
Finland	468	52.07	36.76	35.88	Lundberg & Väisänen, 1979
Czech and Slovak Republics	360	51.18	36.22	34.24	Guthová, 1993
Sweden	319	51.92	36.66	35.59	Svensson, 2002
Sweden	688	51.94	36.57	35.43	Karlsson, 2005
England	69	51.64	36.55	35.19	This study
Lymington - pre-collection	(n = 20)	51.27	36.36	34.57	This study
Lymington - post-collection	(n = 19)	51.79	36.74	35.65	This study
Poole	(n = 20)	51.60	36.46	34.97	This study
Raby Estate	(n = 10)	51.92	36.66	35.59	This study

* Egg volume calculated using the original authors' data for mean length and breadth from each study (as provided in the table), using the Hoyt (1979) calculation described previously (Equation 2.1, Section 2.2.2).

There is very little difference between the data from the sites in this study and datasets that come from a range of sites and from a range of years, suggesting that the eggs collected on the Poole, Lymington (both pre- and post-collection) and Raby sites are fairly representative of the typical black-headed gull egg, being neither particularly long or short, wide or narrow, large or small (in terms of total egg volume). Unfortunately, literature data for individual weights of yolk, albumen and shell, yolk:albumen ratio, eggshell thickness and shell index could not be found for black-headed gull eggs, and thus no comparisons could be made between the eggs of black-headed gulls in this study and those from other areas for these measurements.

2.5 Summary

The results from this study suggest that the eggs from the uncollected Poole colony, and eggs from the Lymington colony prior to collection, are of a higher quality than those from the Lymington colony after collection, i.e. after the birds have been forced to relay several times, in

terms of yolk:albumen ratio. As previously mentioned (Section 2.1.3), a study has been carried out on a number of colonies on the Dorset and Hampshire coast (Wood, 2007; Wood *et al.*, 2009), including those colonies discussed in this study, to examine the effects of commercial collection. The study found eggs from collected colonies to be smaller (in terms of volume) and to have reduced yolk:albumen volumes, compared to those from uncollected colonies. Although in the present study the eggs from Poole and the pre-collection Lymington eggs were not significantly larger than those from Lymington after collection (either in terms of volume or wet weight), the post-collection Lymington eggs had a significantly higher wet weight of albumen, and a lower yolk:albumen ratio than the eggs from the uncollected site, similar to the Wood (2007; 2009) study, and the pre-collection eggs. As previously mentioned, larger eggs often contain relatively more albumen rather than a larger yolk (Romanoff & Romanoff, 1949; Parsons, 1976a; Nisbet, 1978; Ricklefs *et al.*, 1978; Finkler *et al.*, 1998; Lessells *et al.*, 2002), which is considered to play the most important part in determining the health of the embryo and hatchling (Parsons, 1970; Lundberg & Väisänen, 1979; Carey, 1996; Finkler *et al.*, 1998).

The general trend in yolk:albumen ratios indicates that the Poole and pre-collection Lymington eggs had higher average ratios than the Raby eggs, with the post-collection Lymington eggs having the lowest average yolk:albumen ratio. The trend of yolk:albumen ratio decreasing Poole/Lymington Early > Raby > Lymington Late (although not all of these differences are significantly different, statistically), i.e. uncollected/pre-collection > low level collection > high level collection indicates that egg harvesting is having an impact on the intrinsic quality of the eggs. These results are consistent with previous studies in suggesting that the physiological condition of laying female birds is being depleted on commercially harvested sites to a level that affects the size and quality of the eggs produced (Wood, 2007; Wood *et al.*, 2009). However, there are a number of potential confounding factors to consider that may affect the relationship between the site and the egg characteristics, such as the differences between the sites in terms of climate, colony size, level of predation and competition (particularly between the two south coast sites and the northeast inland Raby site), and exposure to pollution. The concentrations of heavy metals and selenium in the egg has the potential to affect the quality of the egg (see Chapter 5), and could be a confounding influence in comparing data from different sites. However, in addition to comparing different sites with different collection regimes, this study has also examined eggs from the same site pre- and post-collection, and has found significant differences between pre- and post-collection eggs in terms of yolk:albumen ratio. The results from this study therefore suggest that the commercial collection of black-headed gull eggs carried out on the Lymington colony is having an adverse effect on the quality of the black-

headed gull eggs, which in turn may have an adverse effect on the breeding success of the birds. This may be attributable to the energy-consuming process of relaying itself, and the effect this may be having on the health of the laying bird, or may be due to an increase in the concentration of pollutants in the egg with relaying. The differences between pre- and post-collection eggs in terms of metals concentrations will be examined in Chapter 5.

CHAPTER 3. IMPACTS OF NESTING DENSITY ON EGG SIZE IN BLACK-HEADED GULLS

3.1 Introduction

Studies were carried out to assess the relationship, if any, between the density of nests in areas of the colony and the size and dimensions of black-headed gull eggs. Data from this investigation provide details as to which areas and conditions are preferable for nesting for black-headed gulls on salt marsh islands, in addition to providing information on the general fitness of the birds in the colony. Eggs collected for contaminant analysis in this project were taken from central, dense areas of the colonies (Section 2.2.1), based on the assumption that the birds nesting in these areas would be of good quality in order to have secured good nesting habitat. This assumption was in turn based on suggestions that black-headed gulls breeding outside the main central colony have reduced breeding success (Patterson, 1965). The sampling strategy employed for egg collection in this study may be validated by the results of this investigation into nesting density and egg size. The fact that one site is commercially collected and the other uncollected could potentially be a confounding influence in determining the effects of nesting density on egg size; however, the sites will be examined separately and the results compared in order to assess the effects of commercial egg collecting on both egg size and dimensions, and on the nesting density. In addition, the results from Chapter 2 show no significant difference between eggs from the collected and uncollected sites in terms of egg size and dimensions, and it is therefore unlikely that the collection regime at the sites would be a significant confounding influence in the assessment of the effects of nesting density on these egg characteristics. However, egg collection may have an effect on the nesting density, and the collection of data from two similar colonies with the major difference being that one colony is commercially harvested enables investigations into the effects of egg harvesting on the nesting density of black-headed gulls, an area of research which has not previously been examined.

3.1.1 Nest density

A number of studies have been undertaken investigating the possible links between nesting density and breeding success in several different species and types of colonially nesting bird. For the purposes of this review, the studies discussed will be limited to those regarding colonial

seabirds, in particular the gulls (*Larus* spp.), which are the genus of concern in this study and are also the birds for which much of the research in this area has been conducted.

The potential complexity of investigating density effects in colonial organisms is reflected in the wide range of results of field studies for seabirds. Studies have produced mixed results, with some reporting no correlation between nesting density and breeding success, others a positive correlation, others a negative correlation, and some reporting a median nesting density as optimal. For example, Birkhead (1977) observed a positive correlation between nesting density and breeding success (indicated by the number of pairs rearing a chick to fledging) with common guillemots (*Uria aalge*), and Becker (1995) investigated breeding success of common terns (*S. hirundo*) in terms of number of eggs in the nest, hatching and fledging success, and found both hatching and fledging rate to be positively correlated with nesting density. In contrast, Houde (1983), also with common terns, found no relationship between nest density and chick survival after accounting for habitat type. Similarly, nest density was not found to be related to chick survival for black-headed gulls (Patterson, 1965), ring-billed gulls (*Larus delawarensis*; Dexheimer & Southern, 1974), Caspian tern (*Sterna caspia*; Antolos *et al.*, 2006) or Western gulls (*Larus occidentalis*; Hunt & Hunt, 1975). A negative correlation between nest density and egg size was found for herring gulls (*L. argentatu*s; Becker & Erdelen, 1986), however, once vegetation variables were included in the analysis (nest density was found to increase with increasing vegetation cover) a significant relationship no longer existed, and the authors concluded that the significance of nest density/egg size correlations was an indirect one mediated mainly by the influence of the vegetation variables. For the great black-backed gull (*Larus marinus*), Butler and Trivelpiece (1981) found egg production and hatching success to be similar in areas of high and low density, but report that gulls in high density areas fledged significantly fewer chicks than those in low density areas. Adults breeding in high density areas were more combative and engaged in significantly more chick-orientated vocalisations than low-density adults, and although the frequency of chick-feeding bouts did not differ, high-density adults regurgitated significantly more food than low-density adults. Parsons (1971; 1976b) observed breeding success in herring gulls (*L. argentatus*) to be highest at median nesting densities. Patterson (1965) studied signs of aggression in black-headed gulls, and found that birds outside the main colony were less aggressive towards a person walking towards their nest, they flew up at greater distances, stayed further away and attacked less. However, the same behaviour was observed in the less dense areas of the main colony and in the main colony on days when fewer birds were present.

In his study with black-headed gulls, Patterson (1965) notes that birds breeding outside the main colony did not produce any young over a two-year study period, and nests towards the edges of the main colony were less successful than those in the centre. However, the relationship between breeding success and location within the colony could not be proven statistically, and studies into nest density effects on breeding success revealed no relationship between the two factors. The study was carried out on a large (8000 pairs) colony nesting on sand dunes on the North coast of England. This type of colony is somewhat atypical, with the majority of black-headed gull colonies nesting on coastal marshes and in pools and reservoirs (Cramp, 1983; Aspinall *et al.*, 1993; Malling Olsen & Larsson, 2004). The sand dune colony studied by Patterson was far more accessible to land predators than the more typical types of colony, and had an exceptionally high mortality rate when compared to 'island' colonies such as marshes. The author himself makes the point that repeat observations in a more typical marsh colony which has lower mortality, and that mainly due to avian predators, would be beneficial before drawing conclusions on the function of nest density in breeding success (Patterson, 1965).

Hunt and Hunt (1975) explain that with colonial nesting comes conflicting needs for protection against nest predation and avoidance of intraspecific aggression. In the absence of interspecific predation, larger territories may confer a reproductive advantage on some laridae due to a decrease in conspecific interference. Indeed, in species nesting where high-quality nest sites are limited (e.g. shags *Phalacrocorax aristotelis*; Potts *et al.*, 1980) and species in which cannibalism is a major cause of egg and chick loss (e.g. great black-backed gulls *L. marinus*; Butler & Trivelpiece, 1981), breeding success tends to be negatively correlated with nesting density. For species where suitable habitat is less limited and cannibalism is uncommon, such as guillemots (*U. aalge*; Birkhead, 1977) and common terns (*S. hirundo*; Becker, 1995), high density generally results in higher breeding success because of reduced predation.

The complex relationship between nest density effects and breeding success in colonial seabirds is reflected in the range of different results previously described. Potential explanations for the variety of effects observed on reproductive success with high- and low-density nesting include asynchrony in the seasonal timing of egg laying and hatching: late breeders have been shown in a number of studies to be less successful than early or peak layers (Paynter, 1949; Patterson, 1965; Antolos *et al.*, 2006), peripheral versus central nesting position in the colony: central breeders tend to be more successful than those on the periphery of a colony (Dexheimer & Southern, 1974; Ryder *et al.*, 1977; Montevecchi, 1978; Brunton, 1997), and age differences between the two groups: some studies have shown immature birds to be less successful breeders and also less able to secure optimal breeding sites (Davis, 1975; Becker & Erdelen, 1986). Such

effects make investigating the effect of nesting density alone on breeding success more difficult and, as previously stated, habitat quality and individual quality are often confounded, making it difficult to separate the effects of individual quality on breeding performance from effects that are attributable to physical components of the breeding areas (Kim & Monaghan, 2005).

Although one study with the herring gull (*L. argentatus*; Davis, 1975) has demonstrated that young birds tend to breed later in the season than older birds, potentially making them less successful breeders, other studies have demonstrated that nesting position in stable colonies is generally unrelated to age (Tenaza, 1971; Nelson, 1978). Provided the colonies studied are long-established and stable it does not therefore appear necessary to determine the ages of the parent birds. Separating out the effects of nest density from nest location in the colony is more difficult: by definition the majority of low-density nesters are found more towards the colony edge (Hutson, 1977).

The variability in the reported results for the relationship (or lack of) between nesting density and reproductive success may be due to methodological, environmental and/or species-specific differences. As mentioned above, only one previous study has been carried out examining the effects of nest density on breeding success of black-headed gulls, and the author reported no significant relationship (Patterson, 1965); more recent studies with this species could not be found, and certain aspects of the Patterson study suggest that the results may not be typical of black-headed gull colonies in general. It is clear that additional data is necessary for clarification, particularly as nesting density can be a confounding factor in scientific studies regarding other aspects of breeding behaviour. This chapter attempts to assess whether or not a relationship exists between nesting density and egg size (which can provide an indication of potential breeding success) in two separate black-headed gull colonies on the South coast of England.

In the context of this study, the eggs and chicks sampled and analysed in Chapters 2, 5 and 6 were taken from nests in the central, most dense part of the colony. This chapter will test the assumption these samples would be those from eggs/chicks produced by birds of comparable fitness, thus eliminating a potential key confounding factor in the analysis in other areas of this study, as well as addressing a controversial issue in avian ecology.

3.1.2 Egg size

Egg size can be used as a measure of reproductive fitness and studies have shown that larger eggs tend to produce larger, heavier chicks with an increased chance of survival over those hatching from smaller eggs (see Section 2.1.1).

As previously mentioned (Section 2.1.1), the size of the eggs in a clutch has been shown to vary with clutch sequence, with the first laid egg being the largest, followed by the second egg, and the third (usually final in gulls) egg being smallest (Paludan, 1951; Preston & Preston, 1953; Parsons, 1972). In this study, therefore, the largest egg will be assumed to be the first laid, followed by the second largest, and the smallest will be assumed to be the third (and final) laid.

3.1.3 Effects of commercial egg harvesting

No literature could be found regarding the impacts of egg harvesting on nest density. The impacts of harvesting on egg size have been discussed previously, with replacement clutches (for example those clutches replaced following harvesting of eggs) reported to contain smaller eggs of lower quality (Feare, 1976b; Parsons, 1976a; Brown & Morris, 1996; Nager *et al.*, 2000; Hipfner *et al.*, 2003; Wood *et al.*, 2009); see Section 1.5.1. This study examined a commercially collected site and an uncollected site, and investigated any effects of egg harvesting on nesting density of black-headed gulls.

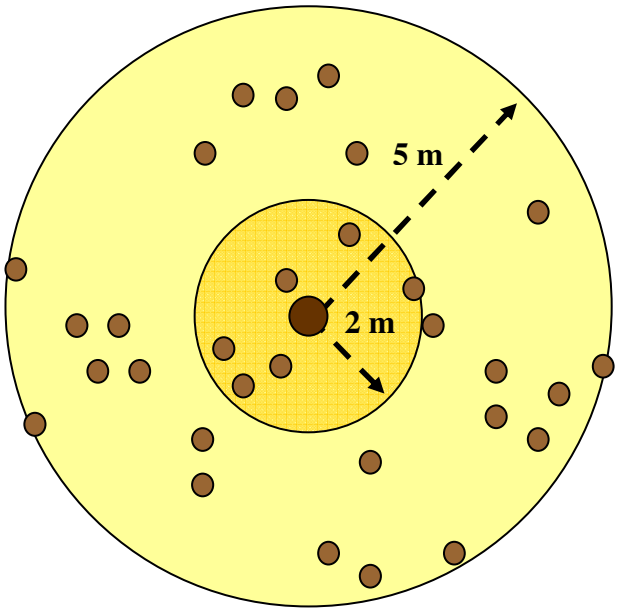
3.2 Methods

During the 2007 breeding season, fieldwork was undertaken to examine the relationship between nest density and egg size of black-headed gulls at Lymington and Poole. The Lymington and Poole colonies were chosen for the nest density analysis as they are similar sites in terms of overall colony size and are both salt marsh island colonies located on the South coast, but have the major difference in that the Lymington colony is subjected to commercial egg collection, whereas the Poole colony is not (see Section 1.6). The Raby site was not included in the nest density studies as the site is very different to Poole and Lymington, being inland and a much smaller colony than either of the coastal sites. The Raby colony is not commercially collected, but is subjected to low-level collection for personal consumption on behalf of the estate owner. By comparing the Poole and Lymington sites alone, conclusions may be drawn not only as to the impacts of nest density on egg quality, but also as to the impact of commercial egg collecting.

Work was undertaken to examine nests in two discrete zones of each colony: the most densely nested zone at the centre of the colony (termed 'centre' nests) and a zone at the edge of the colony where nests were further apart (termed 'edge' nests), determined by eye and confirmed by field measurements. Within each zone 20 nests, each containing a clutch of three eggs, were chosen and marked with a bamboo cane. In order to minimise the effects of timing of breeding and asynchrony of hatching, the reference nests chosen in this study were those containing only complete three-egg clutches, which had therefore been laid within the same time period (rather than those early layers which had chicks or late breeders still with incomplete clutches). These nests were also considered to be from birds laying during the 'peak' laying period, as the majority of nests in the colony contained complete clutches at the time of sampling. For each of the 20 reference nests chosen, nest density ratings were determined by counting the number of active nests (i.e. those containing eggs or chicks) within a two metre and a five metre radius of each of the marked reference nests (see Figure 3-1). Nests on the borderlines of the radii were counted if 50% or more of the nest area was included in the measured radius. Within the reference nest itself, the maximum length and maximum breadth of each egg was measured using vernier callipers (accurate to ±0.1 mm). This method was based on that used in the only previous study that could be found examining nest density and breeding success (Patterson, 1965), in which the authors obtained a nest density rating by counting the number of active nests within a two metre radius of a reference nest. However, in this study a larger five metre radius was examined in addition to the two metre radius in order to assess if any relationship could be found between egg size and nesting density when also assessing a larger area. The nest counts within the radii were converted to nests/m^2 in order to be able to make comparisons between both measurements.

Egg volume was calculated using the formula detailed in Section 2.2.2 (Equation 2.1; Hoyt, 1979), and the relationship between egg size (volume, maximum length and breadth) and nesting density were examined, with the two metre radius, and again for the five metre radius. Results were plotted to examine the relationship between egg size and nest density.

Comparisons were made between the two sites in order to assess whether there were any differences for nest density or egg size with site and for egg size with nest location.



Note: Larger dark brown circle represents the reference nest, smaller light brown circles represent surrounding nests within the two or five metre radii

Figure 3-1 Nest density measurement method showing reference nest and two and five metre radii

3.3 Results

Results of the nest density studies carried out in May-June 2007 are presented below. The number of nests per metre squared within the radii of each reference nest was calculated by dividing the number of nests by the area of the circle (12.6 m^2 for the two metre radius and 78.5 m^2 for the five metre radius).

3.3.1 Egg length and breadth

Maximum egg length and maximum egg breadth were compared with nest densities (nests/m^2) in both the two and five metre radii for the Lymington and Poole sites. The plots in Figure 3-2 to 3-5 show the distribution of egg size in terms of maximum length and breadth, with nest density in a two metre and a five metre radius of the reference nest for the Lymington and Poole sites. No significant trend was observed in this data, as confirmed by correlation analysis (see Appendix A).

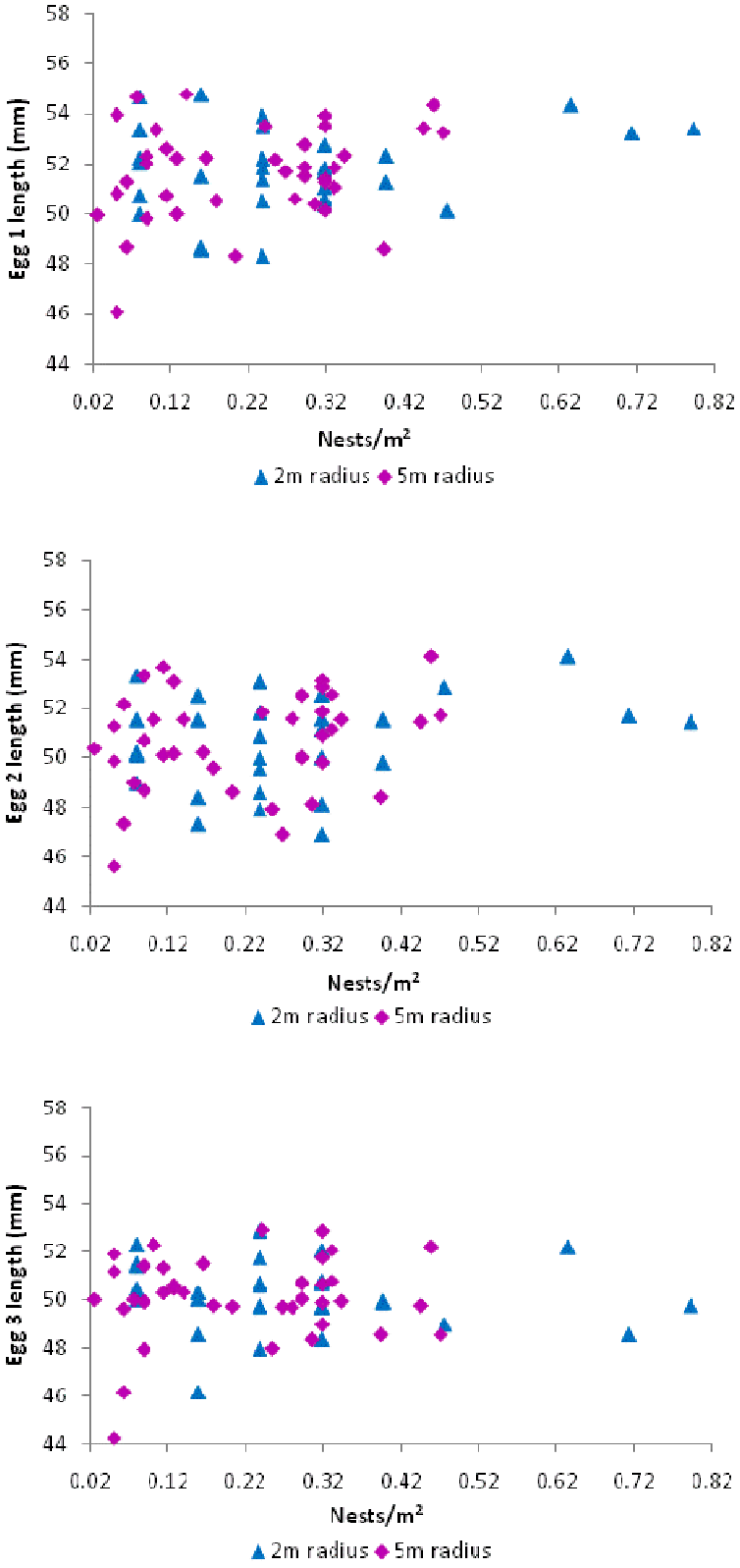


Figure 3-2 Maximum length of black-headed gull eggs compared with nesting density: Lymington site, 2007 (N = 20)

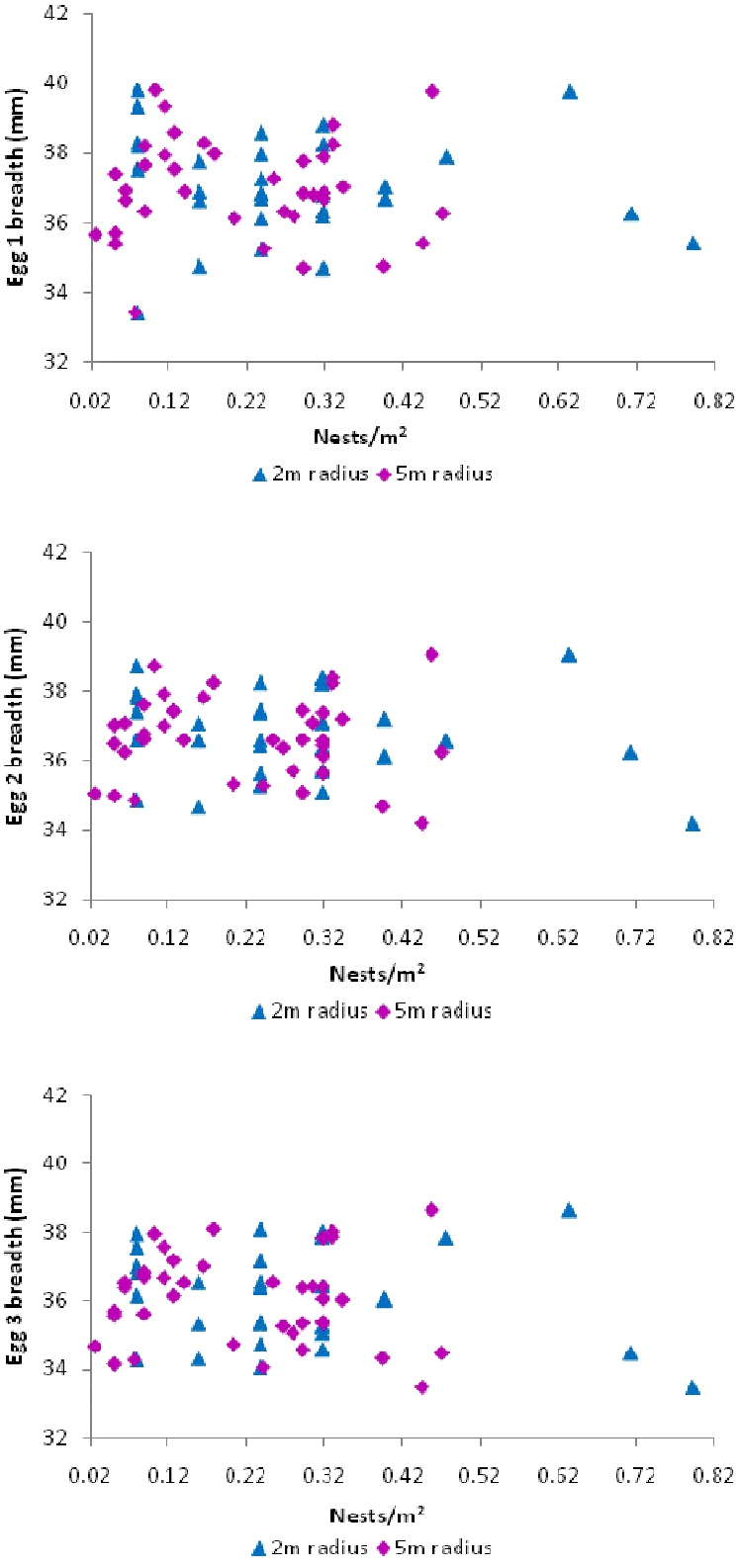


Figure 3-3 Maximum breadth of black-headed gull eggs compared with nesting density: Lymington site, 2007 (N = 20)

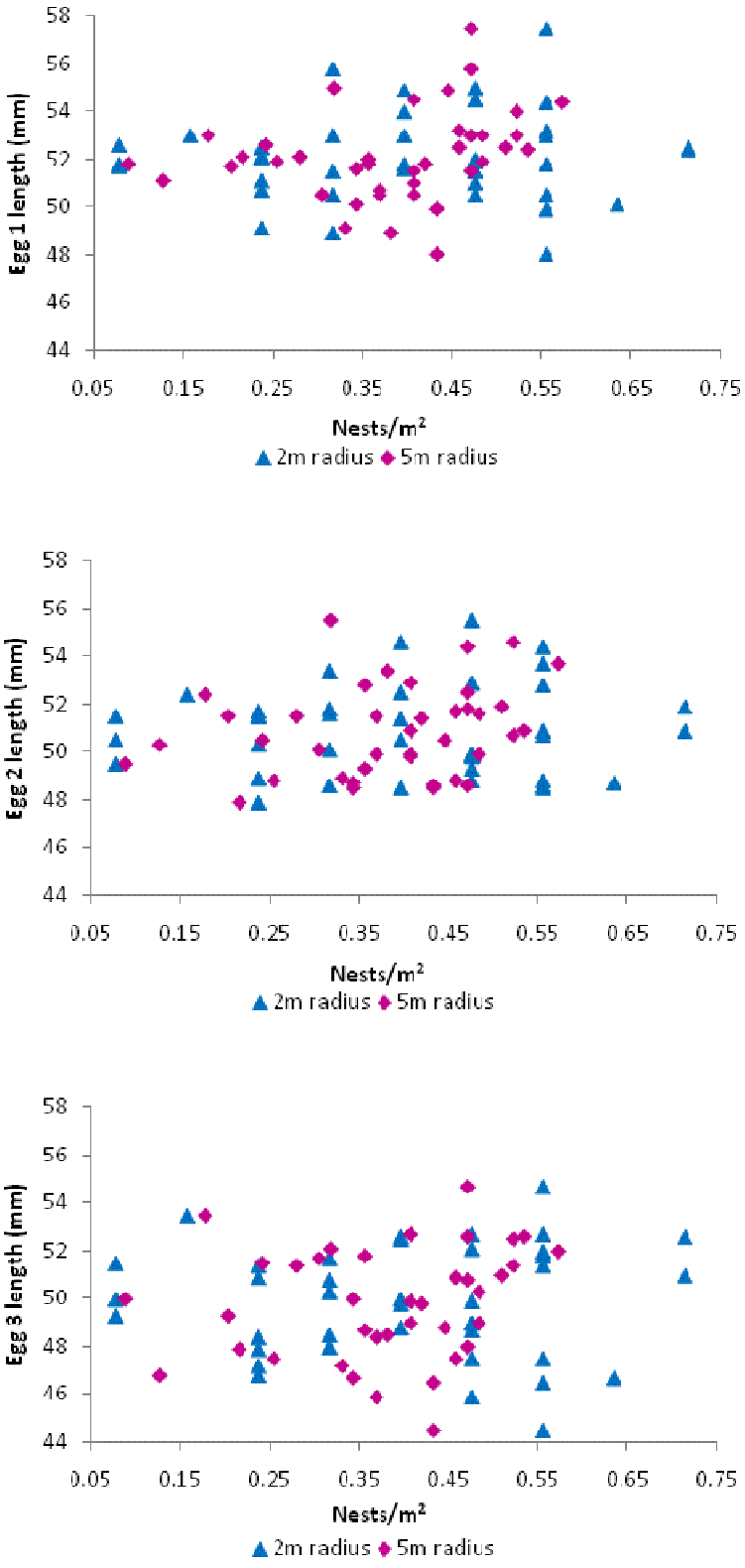


Figure 3-4 Maximum length of black-headed gull eggs compared with nesting density: Poole site, 2007 (N = 20)

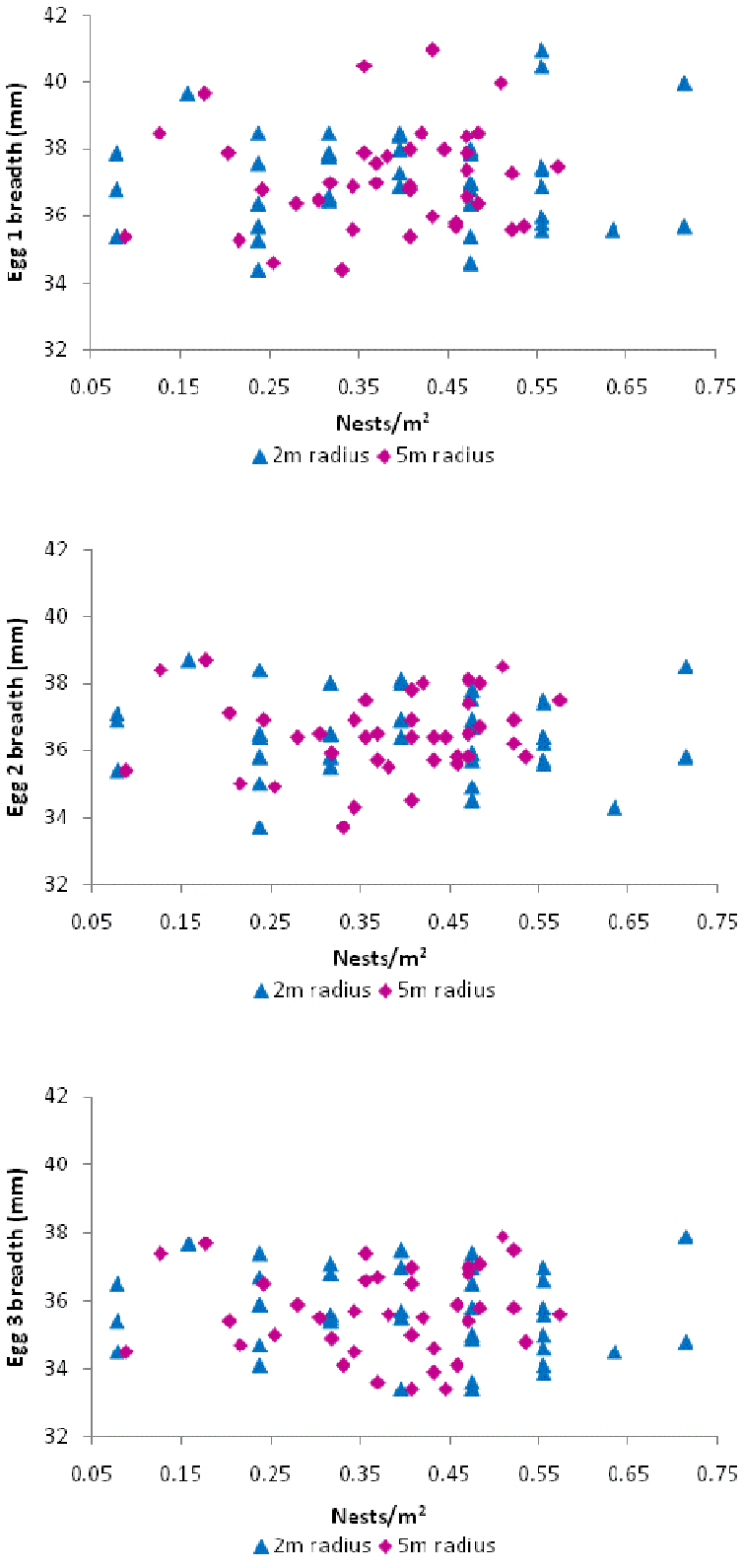


Figure 3-5 Maximum breadth of black-headed gull eggs compared with nesting density: Poole site, 2007 (N = 20)

3.3.2 Egg volume

Egg volume was calculated and compared with the nest density counts (nests/m^2) in both the two and the five metre radius. Figures 3-6 and 3-7 show the distribution of egg volume with nest density in both a two metre and a five metre radius of the reference nest for the Lymington and Poole sites. No significant trends were observed in this data, again confirmed by correlation analysis (see Appendix A).

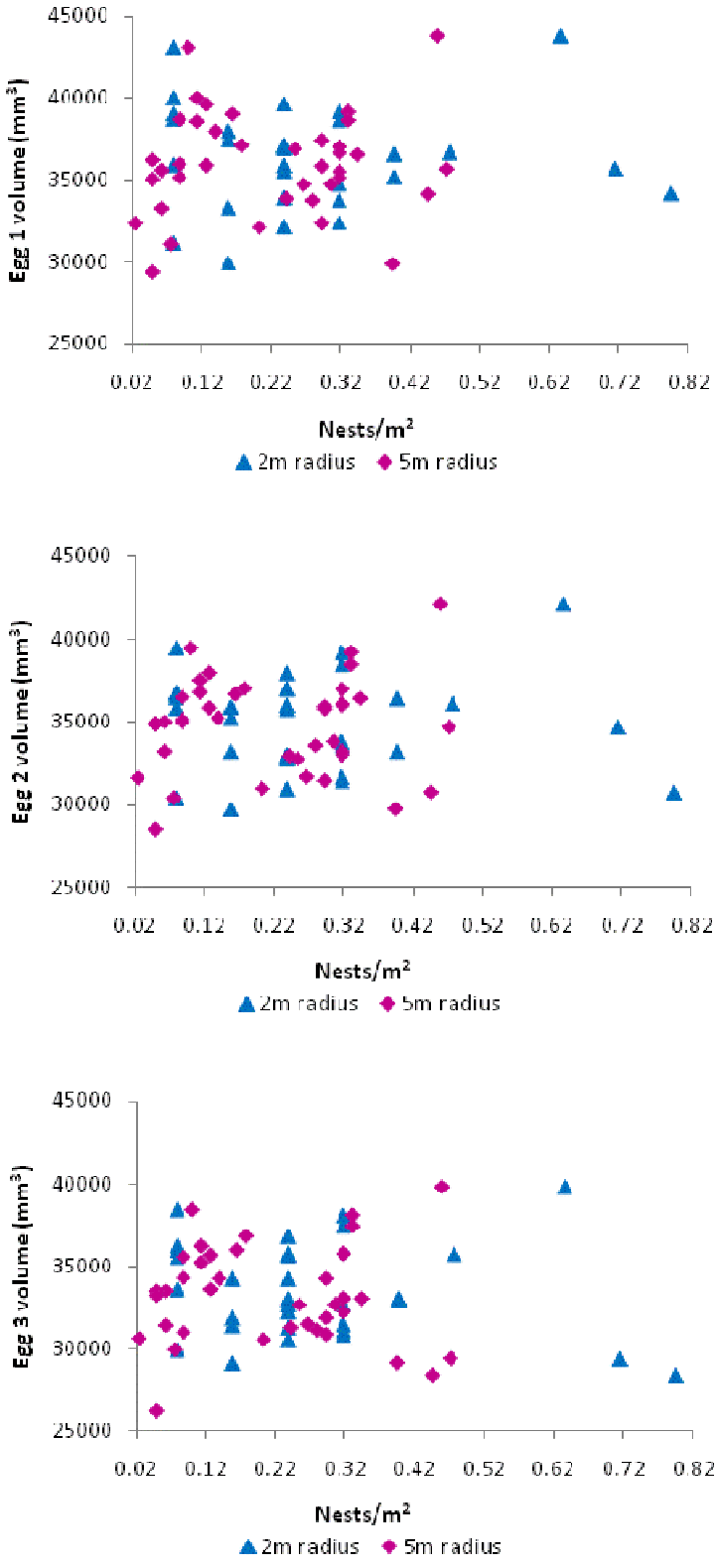


Figure 3-6 Volume of black-headed gull eggs compared with nesting density: Lymington site, 2007 (N =20)

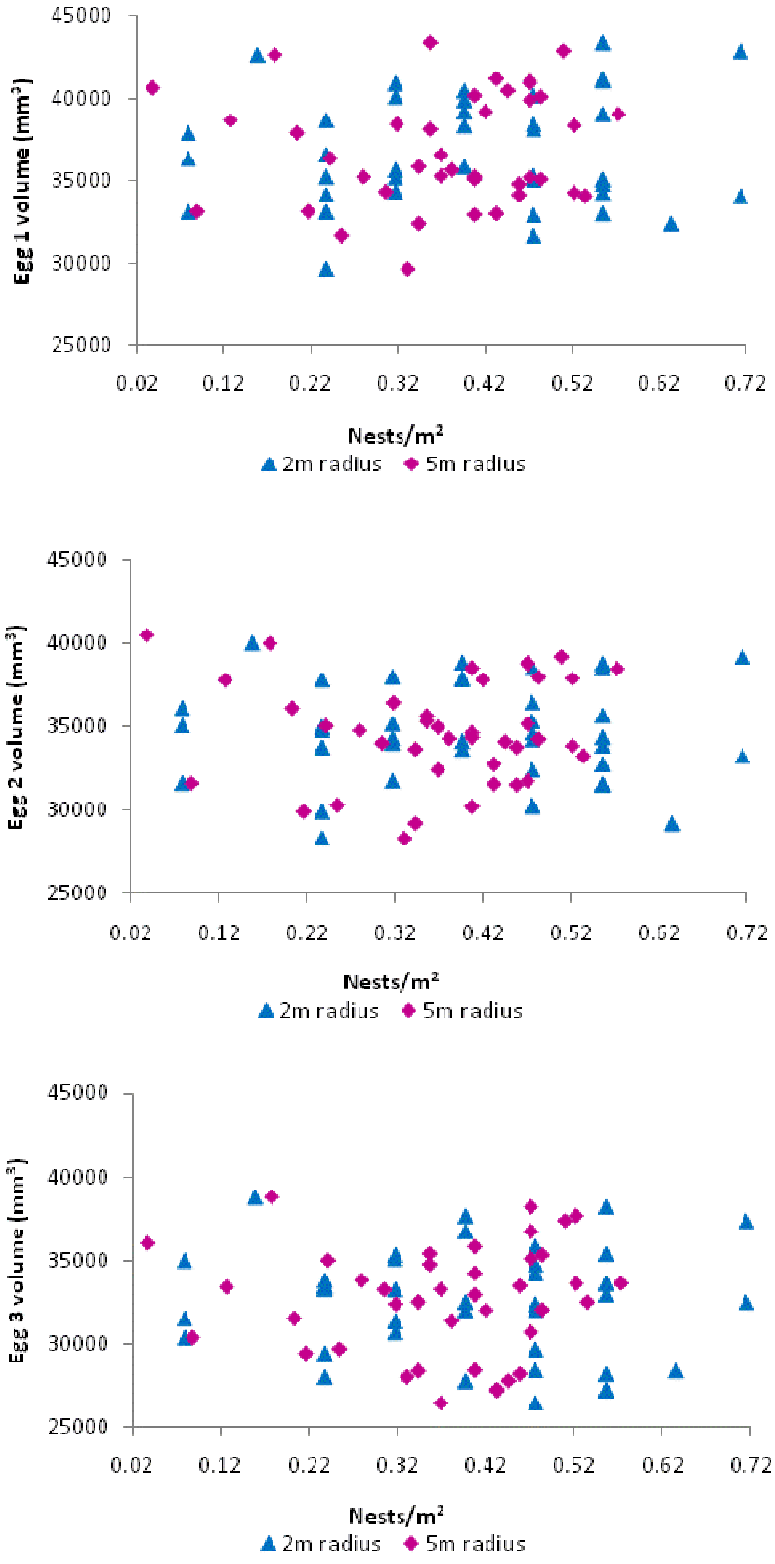


Figure 3-7 Volume of black-headed gull eggs compared with nesting density: Poole site, 2007 (N = 20)

3.3.3 Comparison of nest density and egg size between sites

Pearson's correlation was carried out to examine the relationships between the datasets. No significant relationship ($p > 0.05$) between any of the measurements made/calculated was found for either site, with either the two or five metre densities (see Appendix A), confirming the lack of any apparent relationship in Figures 3-2 to 3-7.

To test for normality, a K-S test was carried out. The K-S test revealed that all data for egg measurements were normally distributed ($p > 0.05$), but the data for nest density in the two and five metre radii were not normally distributed, with the exception of the Lymington data for nest density in the five metre radius, which was marginally above the 5% threshold for significance ($p = 0.024$ and 0.059 for Lymington nest density in the two and five metre radii, respectively; $p = 0.012$ and 0.041 for Poole nest density in the two and five metre radii, respectively). As the distribution of the nest density data is not normal, the criteria for parametric tests are not met and data for nest density will be examined using non-parametric tests. Levene's test for equality of variances was carried out in order to establish that variances were equal.

Comparisons were made between the Lymington and Poole sites to examine any differences between the sites in terms of nest density and egg size. An independent t-test was used to compare data for the two sites and identify any significant difference between the means of the two populations (Townend, 2002), and error plots were produced to examine any significantly different means as indicated by the t-test. However, as the distribution of the nest density data is not normal, the data cannot be reliably tested using the parametric t-test, and was assessed using a non-parametric test - the Mann-Whitney test is a non-parametric test used to test differences between two conditions (Townend, 2002), in this case the two different sites. Results of these tests are provided in Table 3-1.

Table 3-1 Test results for comparison of Lymington and Poole nesting densities and egg size characteristics for black-headed gulls, 2007

Measurement	Mean	S.E.	p	Significance
Nests/m², 2m radius	0.305	0.042	<0.001	**
Nests/m², 5m radius	0.293	0.029	<0.001	**
Egg 1 length (mm)	51.47	0.459	0.650	NS
Egg 1 breadth (mm)	36.74	0.297	0.114	NS
Egg 1 volume (mm^3)	36445	723.7	0.236	NS
Egg 2 length (mm)	50.88	0.472	0.253	NS
Egg 2 breadth (mm)	36.54	0.280	0.366	NS
Egg 2 volume (mm^3)	34746	669.8	0.990	NS
Egg 3 length (mm)	50.38	0.453	0.335	NS
Egg 3 breadth (mm)	36.29	0.343	0.143	NS
Egg 3 volume (mm^3)	32909	694.1	0.353	NS

N = 40. ** = highly significant ($p \leq 0.01$); NS = not significant. S.E. = standard error. Highlighted area indicates results from non-parametric, Mann-Whitney test.

The t-test shows that there is no significant difference between sites for egg length, breadth or volume. The Mann-Whitney test indicates that there are significant differences between the two sites in terms of nest density, within both the two and five metre radii. Error plots were produced in order to examine this relationship further; these are provided in Figures 3-8 and 3-9, below.

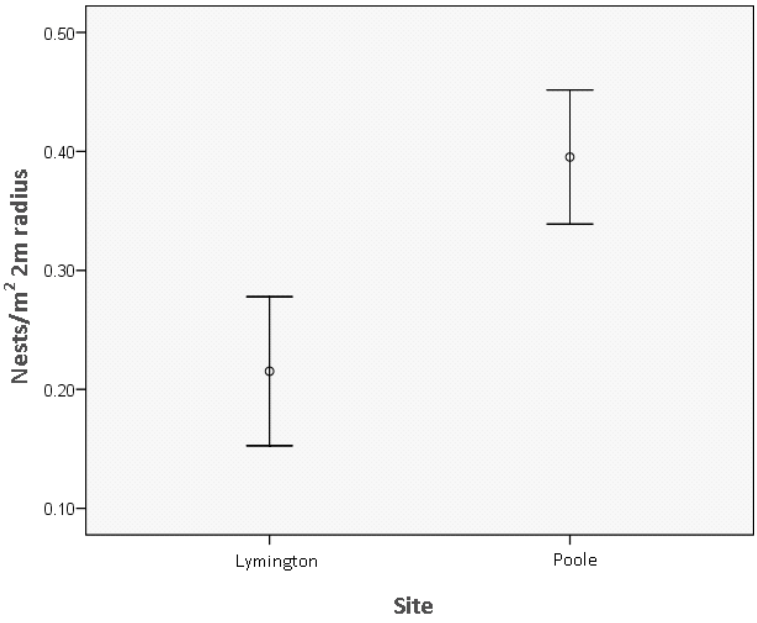


Figure 3-8 **Black-headed gull nest density within a two metre radius for Lymington and Poole sites, 2007 (N = 20); error bars show 95% confidence interval (CI) of mean**

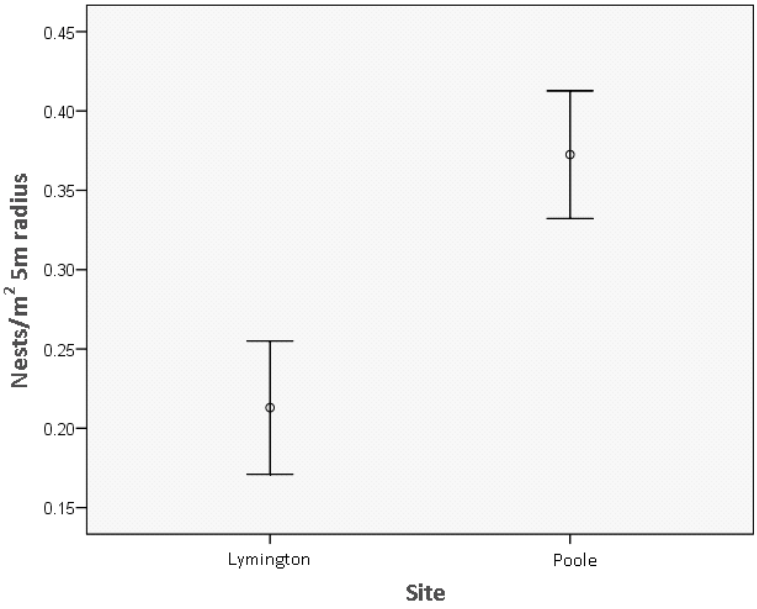


Figure 3-9 **Black-headed gull nest density within a five metre radius for Lymington and Poole sites, 2007 (N = 20); error bars show 95% confidence interval (CI) of mean**

Figure 3-8 and 3-9, combined with the results of the statistical tests, show that the average nest density (taking into account dense, central areas of the colony and less dense, outer areas) within the Poole colony is significantly higher than the average nest density within the Lymington colony, for both the two and five metre radius measurements.

3.3.4 Comparison of egg size with nest location

Comparisons were made between the nests located in the centre of the colony and those located on the outer edge of the colony (see Section 3.2) to examine any differences in terms of egg size. Figures 3-10 to 3-12 provide a comparison of the mean egg length, breadth and volume for eggs in nests located in the centre of the colony with eggs in nests located at the colony edge. Data from both the Poole and Lymington sites were pooled for this analysis to increase the sample size for statistical analysis. As shown in Section 3.3.3, there are no significant differences in any of the egg measurements between sample sites, and thus pooling samples in this way should not affect comparisons of egg size between eggs from central nests and those from edge nests.

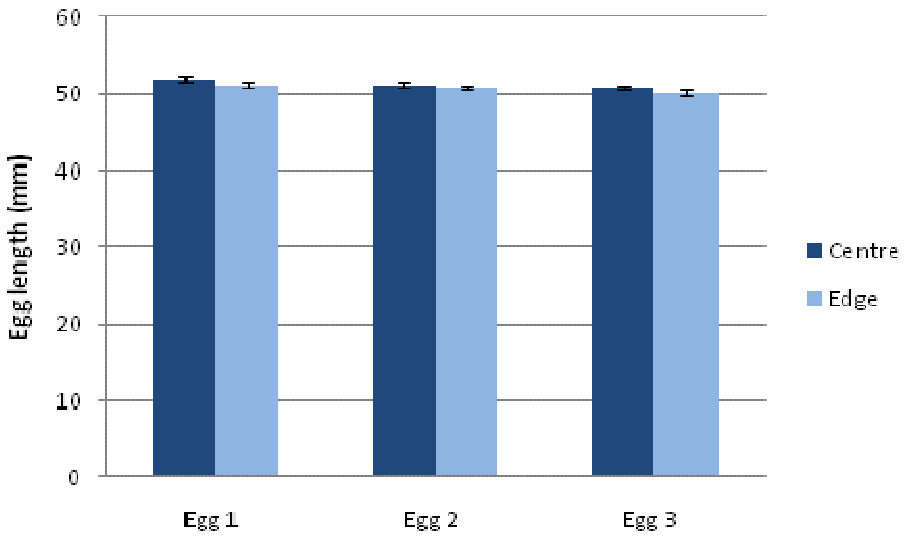


Figure 3-10 Mean length of black-headed gull eggs (mm), ± standard error, from nests located in the colony centre and colony edge, Lymington and Poole sites, 2007 (N = 20)

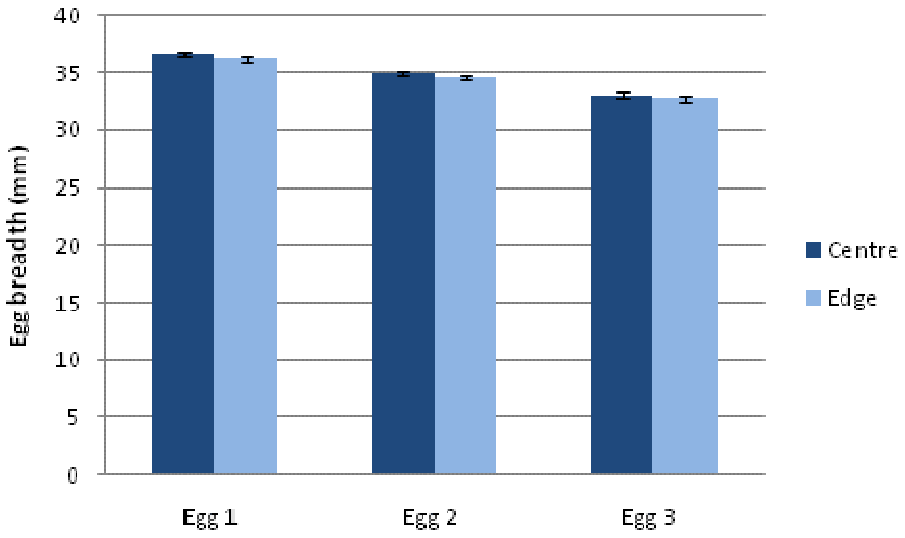


Figure 3-11 Mean breadth of black-headed gull eggs (mm), ± standard error, from nests located in the colony centre and colony edge, Lymington and Poole sites, 2007 (N = 20)

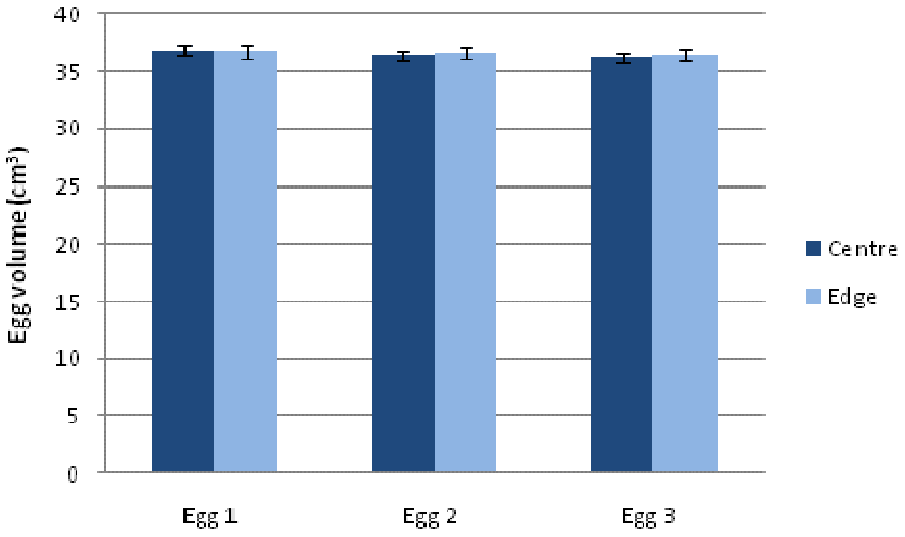


Figure 3-12 Mean volume of black-headed gull eggs (cm^3), ± standard error, nests located in the colony centre and colony edge the colony centre and colony edge, Lymington and Poole sites, 2007 (N = 20)

As all the egg measurement data meet the requirements of parametric tests, an independent t-test is appropriate for examining differences in the data with nest location; results of this test are provided in Table 3-2.

Table 3-2 Independent t-test results for comparison of egg size with nest location (centre of colony vs edge of colony) for black-headed gulls, Lymington and Poole sites, 2007

Measurement	Mean	S.E.	p	Significance
Egg 1 length (mm)	51.46	0.317	0.080	NS
Egg 1 breadth (mm)	36.74	0.213	0.761	NS
Egg 1 volume (mm^3)	36445	513.2	0.625	NS
Egg 2 length (mm)	50.88	0.336	0.463	NS
Egg 2 breadth (mm)	36.53	0.198	0.534	NS
Egg 2 volume (mm^3)	34746	472.3	0.679	NS
Egg 3 length (mm)	50.38	0.320	0.240	NS
Egg 3 breadth (mm)	36.27	0.247	0.978	NS
Egg 3 volume (mm^3)	32909	493.0	0.696	NS

N = 20. NS = not significant. S.E. = Standard error.

The t-test confirms that suggested by Figures 3-10 to 3-12, showing that there are no significant differences with egg size for any of the measurements made/calculated between nest located in the centre of the colony and those located at the outer edge of the colony.

3.4 Discussion

3.4.1 Nest density, nest location and egg size

In a previous study with black-headed gulls, Patterson (1965) investigated the relationship between nest location and breeding success and, based on observations and counts during the course of his study, found that nests outside the main colony failed to rear any young. This observation had also been made by previous workers on the same colony. In the present study, no statistically significant difference was found between nest location and egg size (maximum length, breadth and volume). However, in this study all nests examined were within the main colony and comparisons were made between nests in the centre and those on the outer edges of the main colony, rather than nests actually outside the colony as in the first part of the Patterson

study. The results from the present study are more comparable with the second part of the Patterson study, where the author compared the breeding success, as indicated by number of eggs laid and percentage of chicks fledged, of nests at the edge of the main colony with those at the centre. Patterson (1965) reports reduced breeding success in nests located at the edge of the main colony compared with those in the centre; however, the results were not statistically significant. In the present study, there was no evidence of any pattern in differing egg sizes with nest location (Figures 3-10 to 3-12; lack of relationship confirmed by independent t-test, Table 3-2), and the results from this study suggest that nest location had no effect on the breeding success of black-headed gulls, as indicated by egg size. The results of this study are contrary to those of Patterson (1965); however, although Patterson observed a pattern, the results were not statistically significant. In addition, in the present study egg size was the only indicator of reproductive success measured in this study, whereas Patterson examined fledging success. Breeding success can be measured in a number of different ways, including number of eggs in a clutch, hatching rate, fledging rate, measures of fledgling size and fledgling survival past a set point. In the present study, egg size (maximum length, maximum breadth and volume) was the only indicator of reproductive success measured. Although egg size has been shown to be a good indicator of reproductive success, with larger eggs generally producing larger, heavier chicks at hatching and chicks which have a growth advantage over those hatching from smaller eggs (Parsons, 1975; Nisbet, 1978; Lundberg & Väisänen, 1979; Moss *et al.*, 1981; Birkhead & Nettleship, 1982; Bolton, 1991; Styrsky *et al.*, 1999, and others; see also Section 2.1.1), no evidence was obtained in this study as to the success of hatching, fledging and survival of chicks, and it cannot therefore be concluded that nesting location has no effect on the reproductive success of black-headed gulls at these sites. It can, however, be said that no relationship could be found between egg size and nest location for black-headed gulls nesting on these colonies.

In another part of his nest density study, Patterson (1965) investigated the relationship between nest density and breeding success, making measurements of nest density by counting the number of active nests within a two metre radius of reference nests, within which the number of eggs laid and the percentage of young fledged were recorded, over a time period encompassing two breeding seasons (1962 and 1963 breeding seasons). No correlation was found between density and success in the data from 1962. There did appear to be a positive correlation between the two variables on examination of data from 1963; however, when data were further examined taking into account the correlation between density and laying date, using a partial correlation analysis, the correlation between density and success was reduced and was no longer significant.

Patterson (1965) concludes that the apparent positive correlation between density and breeding success in 1963 was entirely due to the correlation between laying date and density in that year. In the present study, the possible confounding effect of laying date on the results was minimised by selecting reference nests that contained a complete clutch of three eggs. Nests with incomplete clutches were considered to be those of late breeders, and those with chicks already hatched those of early breeders; the majority of nests in the colony contained clutches of three eggs and these were therefore considered to be the nests of birds breeding synchronously at the optimum time. The present study examined a different indicator, and only one indicator, of reproductive success (egg size, versus Patterson's number of eggs and percentage hatching), this study was carried out on more typical salt marsh island colonies (as opposed to a more predator-accessible colony nesting on sand dunes), and examined a wider radius around the reference nest (five metre radius in addition to the two metre radius). However, the findings were the same: no relationship was found between nest density and the indicators of breeding success that were examined.

Species nesting where high-quality nest sites are limited and in which cannibalism is a major cause of egg and chick loss tend to show a negative correlation of breeding success with increasing nest density (Potts *et al.*, 1980; Butler & Trivelpiece, 1981), whereas species for which availability of suitable habitat is less limited and cannibalism uncommon, a positive correlation between nesting density and breeding success has been observed (Birkhead, 1977; Becker, 1995), as a result of reduced predation. Whilst some cannibalism does occur in black-headed gulls - they have been known to take eggs and small chicks of other pairs (Patterson, 1965) and have been noted to "egg-suck", destroying eggs by sucking out the contents (Kirkman, 1937) - intra-specific predation does not appear to be particularly common in comparison to some other species. On the colonies examined in this study, predation is limited owing to their location on marsh islands and therefore fairly inaccessible to mammalian predators such as foxes, rats and so on (Aspinall & Tasker, 1992). However, colonies are still subject to avian predation, for example from great black-backed gulls (*L. marinus*), buzzards (*Buteo buteo*) and peregrine falcons (*Falco peregrinus*). One of the main advantages of breeding in dense areas is the avoidance of predation. The fact that the need for very dense nesting to protect against predation is probably minimised on these colonies, combined with the fact that the need for large distances between nests (i.e. low density nesting) is minimised due to the relatively low level of intra-specific parasitism in this species, may go some way towards explaining why neither a positive nor a negative relationship between nest density and breeding success was apparent. In addition, it should again be noted that only one indicator of breeding

success was measured in this study and therefore it cannot be conclusively stated that nesting location has no effect on the reproductive success of black-headed gulls at these sites, rather that no relationship could be found between egg size and nest location.

Separating out the effects of nest density from nest location in the colony is complicated as the majority of low-density nesters are found more towards the colony edge. A number of authors report reduced breeding success in birds nesting at the far edges of the main colony, and outside of the main colony (Kruuk, 1964; Patterson, 1965; Ryder *et al.*, 1977; Montevecchi, 1978; Spear, 1993). For black-headed gulls, Patterson (1965) observed that nests outside of the main colony failed to rear any young, with few eggs surviving more than 7-10 days and none reaching hatching. Breeding success in areas on the edge of the main colony was also lower than in the colony centre, although these differences were not statistically significant. In the current study, geographical colony edge and colony centre were not defined, although the less-dense areas were almost exclusively located nearer the colony edge and the water's edge than the dense areas, which were more central both geographically and in terms of the colony. Thus it was not possible to eliminate the effects of any potential centre/edge relationship in this experiment. Further study would be prudent, examining not only the eggs in nests from different locations within the colony, but also following up with investigation into hatching success, chick survival and fledging, to provide a more in-depth assessment of the effects of nest location on breeding success.

3.4.2 Effect of commercial egg harvesting on egg size and nest density

The results of this study show that egg size did not significantly differ between the uncollected Poole colony and the collected Lymington colony, which is consistent with the results from the egg dimensions work on different samples from the same colonies (see Chapter 2, Section 2.3). As previously mentioned (see Section 1.5.1), black-headed gulls will relay individual eggs or entire clutches if lost (Weidmann, 1956); however, replacement laying requires high energy reserves and delays breeding (Verhulst & Timbergen, 1991). Studies of egg harvesting have reported reduced breeding success in some seabird populations (Ainley & Lewis, 1974; Feare, 1976a; de Juana, 1984; Haynes, 1987; Vermeer *et al.*, 1991; Burger & Gochfeld, 1994; González, 1999; Shannon & Crawford, 1999; Zador *et al.*, 2005), and egg size has been reported to be reduced in replacement clutches (Feare, 1976b; Parsons, 1976a; Brown & Morris, 1996; Nager *et al.*, 2000; Hipfner *et al.*, 2003). However, it should be noted that this result does not mean that commercial collection is not having an effect on the breeding success of black-headed gulls, rather that egg size does not appear to be affected. Egg size is only one measure of

reproductive success, and no observations were made as to the hatching success of the eggs in the present study, nor the fledging/survival of chicks. Further examination into the hatching and post-hatching survival of chicks would be prudent, in order to further assess the effects of commercial collection on breeding success.

Nest density within the uncollected Poole colony was found to be significantly higher than the nest density within the collected Lymington colony, for both the two and five metre radius measurements. No studies could be found in the literature reporting changes in nest density between harvested and un-harvested colonies. The Poole island colony examined in this study is over twice the size of the Pylewell island colony at Lymington (*ca*. 10000 and *ca*. 4300 pairs, respectively), and the results from this study also show that the Poole colony is more densely populated. The lower nesting densities at the Lymington site may be an indication that this area is less desirable for nesting than the uncollected, undisturbed Poole site, due to the disturbance of the colony by egg collectors, and the heavy collecting of the gulls eggs requiring them to relay several times in order to successfully raise any chicks. In addition to the pressure on the black-headed gull populations nesting on the Lymington marshes as a result of commercial egg collecting, the Lymington islands are far more prone to flooding (Wood, 2007) than the islands located in the much more sheltered Poole Harbour; flooding leads to loss of eggs and young chicks, which may make the Lymington salt marshes less desirable habitat that the Poole salt marshes. Loss of habitat due to the rapid erosion of the salt marsh islands that the gulls nest on may also be a contributing factor; both the Poole and Lymington marshes are eroding, however, the salt marsh at Lymington is eroding more rapidly than the salt marsh at Poole, with studies on the loss of salt marsh suggesting that the marsh at Lymington is eroding at almost twice the rate of the marsh at Poole (SCOPAC, 2004a; SCOPAC, 2004b). This loss of habitat may also make the Lymington salt marshes a less desirable nesting location than the salt marshes at Poole.

3.5 Summary

This chapter has shown that nesting density and nest location (centre of the colony vs colony edge) do not significantly affect the size and dimensions of black-headed gulls on the colonies examined. Although the fact that one site is commercially collected and the other uncollected could potentially be a confounding factor in determining the effects of nesting density on egg size, the results from this chapter have confirmed the findings of Chapter 2, with no significant effect on egg size and dimensions as a result of commercial egg collecting. However, the results

have shown that the overall density of nests on the commercially collected Lymington site is significantly lower than the density of nests on the undisturbed Poole site.

In assessing the results of this study, it is important to note that only one measure of breeding success was examined in this study - egg size - and it cannot therefore be conclusively said that nesting density, nest location and commercial egg collection do not have a significant impact on the breeding success of black-headed gulls, rather that these factors do not appear to have a significant impact on egg size. Indeed, the present study has shown that the intrinsic quality of the eggs (indicated by yolk:albumen ratio) on the collected Lymington site is significantly lower that those from the uncollected site (Chapter 2), and previous studies on the same colonies by Wood *et al*. (2009) have shown the number of failed eggs to be significantly higher for the collected Lymington colony, compared with the uncollected Poole colony.

CHAPTER 4. POTENTIAL SOURCES OF POLLUTION AROUND THE LYMINGTON, POOLE AND RABY SITES

This chapter provides information regarding the main potential sources of pollution in the area around the Lymington, Poole and Raby sites, to which the black-headed gull populations examined in this study may be exposed. Birds are exposed to metals in a number of ways: via their food and water, through the cleaning of their feathers, respiratory exposure to airborne contaminants, dermal absorption while swimming and through contact with excrement of other birds, and direct contact with anthropogenic waste products such as landfill waste, chemical, oil and fuel spills (see Section 1.1). Ingestion of food and water the main routes of exposure for seabirds and, because seabirds feed at the upper trophic levels of ecosystems, they can be exposed to relatively high concentrations of contaminants in their prey and can provide information on the extent of contamination in the whole food chain. However, bird behaviour, in particular their mobility and migratory habits, can render birds much less suitable as biomonitors of local pollution as it can be difficult to determine the spatial scale they represent (Furness *et al.*, 1993) and exactly when and where exposure occurred. As previously mentioned, breeding females spend many weeks on the breeding grounds before laying, acquiring sufficient resources (and contaminants) locally to produce eggs (Burger, 2002), thus the body reserves of breeding gulls, and any associated contaminants, are likely to have been acquired from within a reasonably localised area. In this review, the 'local area' within which potential sources of pollution are examined is taken as the area within an 18.5 km radius of the sampling sites, in accordance with a study with black-headed gulls that found the maximum foraging distance during breeding to be 18.5 km (Gorke & Brandl, 1986; see Section 1.1). Basing the review of potential pollution sources within this 'local area' works on the assumption that. However, although black-headed gulls usually feed at distances no greater than 18.5 km from the nesting site immediately prior to and during breeding, there is still potential for body reserves built up over the long-term to be used in the production of eggs, and it is important to note that the potential sources of pollution reviewed in this chapter may not represent a complete assessment of all sources of pollution to which the birds may have been exposed.

The sources and release of metals as a result of local geological influences, land use (roads, agricultural land, urban areas and so on) industry and waste (landfill and sewage), and boats and shipping activities are covered in this chapter, encompassing the main sources of metal pollution to the environment. Inputs to each area from rivers and streams will also be considered. The

environmental fate of each of the individual metals will be discussed, and the impacts of the emissions highlighted in this chapter will be further examined and related to the metal concentrations found in black-headed gull eggs in Chapter 5.

4.1 Geology

Local geological influences in an area can be important in terms of natural sources of metals. Because of the enrichment of trace metals and intensive weathering, the influence of the parent rock on the total content of trace elements in surface soils is related to the processes of soil formation, which may lead to the mobilization and redistribution of metals within the neighbouring soil types (Thornton & John, 1980). Thus, the parent material is the most important factor in determining heavy metals naturally occurring in soils (Zhanga *et al.*, 2008).

Granite (a hard igneous rock) is the parent material that tends to be most associated with high concentrations of heavy metals including arsenic (Peters & Blum, 2003), copper (Ahmad, 1977), lead (Bjorlykke & Thorpe, 1982), nickel (McGrath, 1995), vanadium (Lide, 2008) and zinc (Kiekens, 1995). Owing to the association with heavy metals, areas with underlying granite have been heavily mined for metals in the UK in the 19th century, particularly in the north of England. The erosion, transport and deposition of historically contaminated material is a very important source of sediment-borne metals in all mining-affected river systems in England and Wales (Macklin, 1992). Particular metals of concern resulting from historic mining activities are arsenic, copper, lead manganese, selenium and zinc (WHO, 1981; Hudson-Edwards *et al.*, 1997; Johnson & Younger, 2005; Environment Agency, 2008).

Metals associated with sedimentary rock parent materials such as sandstone, limestone and shale, include cadmium (Page *et al.*, 1987), copper, selenium and zinc (Alloway, 1995), with selenium and zinc more abundant in shales and clay than in limestone or sandstone (Alloway, 1995; Zhu & Zheng, 2001). Iron, being the second most abundant metal on earth, is associated with all soil, rocks and minerals (Cotton *et al.*, 1999).

4.2 Land use

Diffuse pollution is closely linked to land use. Natural waters are impacted by runoff from agricultural land following rain or land drainage, which may lead to pollution of waters with pesticide residues, fertilisers and farm animal waste (Drake, 2007). Fertilisers commonly used

in agriculture may contain cadmium, copper, manganese, vanadium and zinc, and pesticides, herbicides and fungicides used to treat crops and animal stock commonly contain arsenic, copper and selenium (Denton *et al.*, 1997). Animal waste can also be a significant source of heavy metals to groundwaters and agricultural runoff, with faeces containing nickel (Moriyama *et al.*, 1989), and the use of veterinary medicines, dietary and growth supplements in livestock farming leading to release of arsenic, cobalt, copper, iron, manganese and selenium in animal waste (Denton *et al.*, 1997). Urban developments also create diffuse pollution through increased surface runoff, and have potential to contaminate watercourses with heavy metals. Runoff from urban areas is extensive owing to the impaired drainage in built-up areas, and commonly contains heavy metals such as cadmium, copper, lead and zinc (Sea Grant, 2009), the majority of which originates from the use of motor vehicles.

It has been well documented that vehicles are a major contributor to heavy metals in vehicle dust and hence urban and road runoff (Comber & Gunn, 1996; Legret & Pagotto, 1999; Rule *et al.*, 2006) and evidence for higher metal concentrations in runoff associated with roads used by commercial traffic has also been reported (Dannecker *et al.*, 1990). Roads, road bridges, parking areas and other impervious surfaces create diffuse pollution through increased surface runoff, and are subject to small spills, leaks and other emissions from automobiles and other equipment (Hewett, 2003). On the road surface, most heavy metals become bound to the surfaces of road dust or other particulates, and the bound metals will either become dissolved or be swept off the roadway with the dust when it rains. In either case, the metals enter the soil, local water bodies or are channelled into a storm drain, which usually discharge into a nearby water body (Sea Grant, 2009). The main vehicle-related emissions of metals are through particles from exhausts as a result of fuel combustion (cadmium and vanadium), fuelling processes and leaks (lead and nickel), wear of brake linings (copper, nickel and zinc), and tyre wear (cadmium and zinc; Legret & Pagotto, 1999). Although the use of leaded petrol has steadily declined since the 1980s, it should be noted that 'unleaded' fuel is actually fuel without lead additives, and petrol still contains lead as an impurity; thus lead emissions still result from fuel combustion, although to a lesser degree than from previously used 'leaded' fuel (Pacyna *et al.*, 2007). Car washing has also been implicated as a major source of heavy metals entering the surface water system, particularly for zinc, lead and cadmium (Sörme & Lagerkvist, 2002). Motor oil and grease accumulate metals as they come into contact with surrounding parts as the engine runs, and thus oil leaks become another pathway by which metals enter the environment, particularly zinc and nickel (Makepeace *et al.*, 1995). Wear of bearings and other engine parts

is a source of copper, iron and lead, rust and corrosion of galvanised parts are sources of iron and zinc, respectively (Sörme & Lagerkvist, 2002).

4.3 Industry and waste

Metals are released as a result of many industrial processes and may be released directly to natural waters or via surface water runoff. Metals such as mercury, cadmium, lead, and copper from industrial sources are common contaminants of salt marshes and estuaries (Sanger *et al.*, 1999), although the specific contaminants released are dependant on the industry type. Many industries also produce wastewater which goes to sewage treatment works for treatment prior to release to natural waters, in addition to solid waste which is sent to landfill. Sewage treatment works receive wastewater from commercial and urban areas and industry, which is treated prior to release to natural waters. Although this wastewater is treated to remove chemical contaminants and other harmful substances, release from sewage treatment works can still be a significant source of metals and other contaminants to natural waters. In addition, black-headed gulls are frequently found scavenging at sewage treatment works, as well as sewage outlets (Cramp, 1983), and thus sewage treatment works can provide a direct source of metals to the diet through contamination of food sources exploited on the site.

As well as ongoing discharges from industry, it is important to consider the historical industry in an area. Although no longer active, past industrial releases can leave a legacy of historical pollution in sediments and soils for many years. Heavy metals have been shown to accumulate in sediments and soils (Humphreys & May, 2005; Drake, 2007); these metals can accumulate in organisms that live within the sediment and soil, which are an important food source for black-headed gulls (see Section 1.5), and may then be bioaccumulated up the food chain (Langston *et al.*, 2003). Many metals in sediments may also be remobilised, and thus become bioavailable, as they are oxygenated through disturbance during dredging, strong tides or storm events, bioturbation by benthic organisms or erosion.

Landfills can be significant sources of metals to natural waters via surface runoff: waste decomposition and percolation of rain water through waste stored in the landfill results in contaminated liquid known as landfill 'leachate' (Taulis, 2005). As water passes through the landfill, it may 'leach' pollutants from the disposed waste to areas deeper in the soil (Saleem, 1999). However, in recent years landfill technology has evolved from open, uncontrolled dumps to highly engineered facilities designed to eliminate or minimise the potential adverse

impact of the waste on the surrounding environment and, particularly since the introduction of the Landfill Regulations in 2003, leachate is contained and treated before release to the sewer or natural waters and discharges are regulated (Environment Agency, 2010). Perhaps more significant, in the case of this study, is the fact that landfill sites are frequently exploited by gulls as a source of food.

Depending on the type of waste received, landfills may hold many waste items containing heavy metals and selenium. The Environment Agency classify landfills by the type of waste they receive, with categories including 'Household, Commercial and Industrial Waste', 'Industrial Waste', 'Non-biodegradable Wastes (non construction)', and 'Inert Waste (construction and demolition)' (Environment Agency, 2009a). Household, commercial and industrial waste, and inert construction and demolition waste, may contain many materials with the potential to leach heavy metals and selenium into surrounding ground and surface waters. Old paint cans and other containers made of metal, as well as remnants of paint with pigments are usually composed of metal compounds and are a major source of lead and zinc (Comber & Gunn, 1996; Davis & Burns, 1999; Davis *et al.*, 2001). Stainless steel and other metal alloys and protective coatings have numerous uses in domestic appliances such as washing machines, kitchen sinks and so are an important source of metals, including cadmium, iron, lead, nickel, selenium, vanadium (Comber & Gunn, 1996; Denton *et al.*, 1997; Rule *et al.*, 2006). Metals also have many uses in electronics, wiring and electroplating, thus discarded electrical items such as televisions, radios and so on are potential sources of cadmium, cobalt, copper, selenium and zinc (Comber & Gunn, 1996; Denton *et al.*, 1997; HSDB, 2009d). Discarded batteries are another potential source of cadmium, lead and manganese (Eisler, 1985a; HSDB, 2009a; HSDB, 2009e). Arsenic, copper and zinc are used in wood preservatives, and cobalt, iron, manganese, selenium and zinc in pigments, inks and varnishes (Denton *et al.*, 1997); thus, any waste materials comprising treated wood or inks and pigments is a potential source of these metals. Remnants of discarded fuel oil or petrol are a source of manganese, nickel and zinc, and discarded tyres and synthetic rubber-containing products are an important source of vanadium and zinc (NAS, 1979; HSDB, 2008c). Glass and ceramics contain arsenic and cadmium (Denton *et al.*, 1997). Of particular importance in household waste is the presence of zinc in numerous personal care products such as cosmetics, sun blocks, deodorants, shampoos and many other products (Comber & Gunn, 1996; Rule *et al.*, 2006).

Industry, sewage treatment works and landfill all potentially release heavy metals and other contaminants to air as well as to water. Although initially released to air, heavy metals and other pollutants are removed from the atmosphere by dry deposition and wet deposition. Wet

deposition occurs mainly in upland areas where rainfall is highest, and pollutants are deposited in rain, snow and fog; dry deposition is greater than wet deposition in many parts of the UK, and is the term for when 'dry' gases and particles are deposited directly on to the land (Environment Agency, 2009b).

4.4 Boats and shipping

Recreational boating, port operations and commercial shipping all have the potential to impact harbour waters in a number of ways, and metals and metal-containing compounds have many functions in boat operation, maintenance and repair. Potential sources of metal pollution arising from boats and marinas include sewage (pumped treated or untreated directly from boats) and contaminated bilgewater; metal-containing paint particles from sandblasting, boat washing and general erosion (cadmium, copper, lead and zinc; Comber & Gunn, 1996; Davis & Burns, 1999; Davis *et al.*, 2001); metal shavings from engine oils and worn metal parts; fuel combustion (lead and vanadium; Legret & Pagotto, 1999; Laden *et al.*, 2000); spillage, leaks or incorrect disposal of chemical cleaners, pesticides, fungicides, algicides and wood preservatives and varnishes (arsenic, cobalt, copper, iron, manganese, selenium and zinc) applied to the hulls of boats (Scheuhammer, 1987; OhioEPA, 1995; NOAA, 2007). Exhaust emissions from ship funnels contain lead, nickel, vanadium and zinc (Tillman, 1994; Isakson *et al.*, 2001), which are released to atmosphere and subsequently deposited onto land and into water by wet and dry deposition. Small amounts of fuel and oil also enter the marine environment directly as a result of both commercial shipping and recreational boat use and, although amounts are small and release sporadic, this incremental pollution adds up to hundreds of thousands of gallons globally every year (Olsson, 1999) and is a potential source of lead, nickel and vanadium (Al-Swaidan, 1996; Legret & Pagotto, 1999; Laden *et al.*, 2000). Fuelling operations and the repair and maintenance of engines have the greatest potential of contributing to fuel and oil pollution, particularly if waste is not properly managed (Hewett, 2003). Marinas, terminals and areas around fuelling/maintenance points may therefore be most susceptible to an accumulation of these small spills.

Perhaps the most significant heavy metals associated with boats and shipping are copper, tin and zinc, owing to the use of anti-foulant paints and sacrificial zinc anodes. Most paints used on boat hulls and other underwater structures are made with chemicals and biocides designed to leach out and prevent fouling of the portion of the hull below the waterline by marine animals and plants that would otherwise adhere to it. The biocides in these anti-foulant paints include

inorganic substances, organometallics and organic compounds (Comber *et al.*, 2002). Historically, one of the principal components in these products was organotin; however, following restrictions on the use of organotin-based antifoulants, paint manufacturers have produced products using copper and copper oxide, and many antifoulant paints also contain zinc compounds used as 'booster biocides' to optimise the efficacy of the standard antifoulant paints. Although copper and zinc are generally believed to be less toxic than the previously used organotin compounds, high levels can still cause harmful effects to marine life and copper becomes toxic at concentrations only 10-50 times the concentrations required for normal growth and survival of organisms (Hall & Anderson, 1999). As the harmful substances in these paints are designed to leach out over time, they will enter the aquatic environment on a constant basis. In addition, concentrated amounts of these substances may be released during hull maintenance and repair areas. A survey of chandlers, paint manufacturers and boat owners showed that up to 300 tonnes of copper and 0.5 tonnes of zinc pyrithione are used each year in the UK (Boxall *et al.*, 2000). Despite measures to control waste from boatyards and associated activities, a proportion of this waste will inevitably enter the aquatic environment. Zinc is also commonly used in sacrificial anodes to provide corrosion protection for a number of marine applications such as fishing and leisure boats, rudders, outboards, offshore platforms and oil pipelines, resulting in local water contamination by the solubilised zinc (Bird *et al.*, 1996; Rousseau *et al.*, 2009). In harbours, the increasing numbers of ships using zinc sacrificial anodes, combined with the lower renewal of the water from tidal inputs, contributes to an increase of the water zinc level (Bird *et al.*, 1996).

A further important source of metal contamination associated with boats and shipping is the practice of dredging. Concentrations of heavy metals in sediments usually exceed those in overlying water by between three and five orders of magnitude (Bryan & Langston, 1992), and sediment-bound metals can become remobilised, and thus bioavailable, as a result of dredging activities. Oxygenation of sediments during dredging will result in certain metals becoming oxidised and solubilised, initially, before becoming bound to sediment again (Langston *et al.*, 2003). In most ports, maintenance dredging (i.e. removal of sediment that has built up in existing channels or basins that have previously been dredged) is necessary to keep navigational channels open and maintain access to marinas (Drake, 2007).

4.5 Lymington Estuary

4.5.1 Geology

Gravel, sand and clay predominate in Lymington and the surrounding area, with underlying chalk and some limestone also present (Barnes, 2009; Natural England, 2009; New Forest NPA, 2008); the rocks and soils of the area are unlikely to be a significant source of heavy metals to the environment when compared with anthropogenic sources in the area.

4.5.2 Land use

Urban areas and roads

The largest urban areas around Lymington are the city of Southampton (population *ca*. 322000) and its suburbs, and the towns of Christchurch (population *ca*. 40000), Ringwood (population *ca*. 15000) and Lymington itself (population *ca*. 14000) on the mainland, and Newport (population *ca*. 24000) and Cowes (population less than 10000) on the Isle of Wight, in addition to many small villages (Information Britain, 2009).

The major roadway in the area around the Lymington site is the M27 motorway to the north, which becomes the A31 dual carriageway. A number of smaller A-roads run through the New Forest and around the Isle of Wight, in addition to many connecting B-roads, all of which will contribute to the heavy metal pollution of soils, groundwater, rivers and estuaries through road runoff (see Section 4.2). There are also many car parks around the area, 16 of which are situated on the coast; however, most notably there are two large public car parks situated right alongside Lymington Estuary, to provide parking for visitors to the Seawater Baths and The Quay (New Forest District Council, 2004a), where black-headed gulls can be observed scavenging for scraps of food left by tourists (*personal observation*).

The British Petroleum (BP) oil terminal at Hamble-le-Rice (Map ref. 20, Figure 4-1. below) is a fuels storage and distribution terminal which receives fuel from refineries, including crude oil from the Wytch Farm oilfield at Wareham, Dorset via an underground pipeline (Drake, 2007). A major part of the terminal's operations is road tanker deliveries, which take place on a round the clock basis with petrol and other products being supplied to petrol stations, industrial, commercial and domestic customers across southern England (Hamble Interactive, 2009). Although no direct emissions of heavy metals to the environment as a result of the operation of

this terminal have been reported in recent years (Environment Agency, 2009a), the increased traffic as a result of tankers going to and from the facility, combined with the potential for minor fuel and oil spills, is likely to lead to increased concentrations of heavy metals in runoff (see Section 4.2).

Southampton international airport is located just outside the city of Southampton. The airport has one runway of just over 1.7 km in length, and handles an average of just under two million passengers per year (CAA, 2009). Although no point source emissions are reported from the airport (Environment Agency, 2009a), emissions to air as a result of fuel combustion in plane engines and runoff from the runway are likely to be a source of heavy metals, and there is potential for minor fuel and oil spills during refuelling operations (see Section 4.2). In addition, the airport generates increased traffic in the area.

Farming and agriculture

The main rural area within the area of interest around the Lymington site is the New Forest, which is dominated by woodland (both managed and natural) and heathland (Environment Agency, 2006). Around a quarter of the New Forest National Park is farmland, with nearly 60% of this being permanent grassland reserved for grazing livestock and around 20% used for growing arable crops (New Forest District Council, 2010). The water around the New Forest coast is not considered to be at particular risk from agricultural runoff (New Forest District Council, 2004a).

Rivers and riverine inputs

The only river draining directly into Lymington estuary is the Lymington River. The Lymington River and its three main tributaries - the Highland Water, Blackwater and Oberwater - all originate in the New Forest. The rivers join together and then flow south-eastwards through some small villages, the largest of which is Brockenhurst, with a population of approximately 6000 (Information Britain, 2009). The river then flows through the small town of Lymington and continues south to enter Lymington Estuary. As the catchment is largely National Park woodland and heathland with some agricultural land, Lymington River and its tributaries are more likely to be impacted by runoff primarily from agricultural land, rather than urban runoff.

Beaulieu Estuary, Christchurch Bay and Southampton Water are also within the feeding radius of a breeding black-headed gull. The main rivers draining into these areas are the Beaulieu River into Beaulieu Estuary, the River Avon into Christchurch Bay, and the Rivers Test, Itchen and Hamble into Southampton Water. The River Medina also enters the Solent at Cowes on the Isle of Wight. In terms of input from runoff, Southampton Water is likely to be most significantly affected by urban runoff, due to its proximity to the city of Southampton and its suburbs.

4.5.3 Industry and waste

Figure 4-1 shows the potential sources of industrial pollution within an 18.5 km radius of the Lymington site (shown by the black circle). The pollution sources marked include waste (landfill, sewage and waste treatment) and industry (including fuel and power, metal, mineral and chemical industries) operations. The map and information therein have been compiled from information on the Environment Agency 'What's in Your Backyard?' website (Environment Agency, 2009a). A full list of the sites marked on this map is provided in Appendix B, Table B.1. For the purpose of this review, only those sites reported to release the metals of concern in this study to air or natural waters will be discussed in detail.

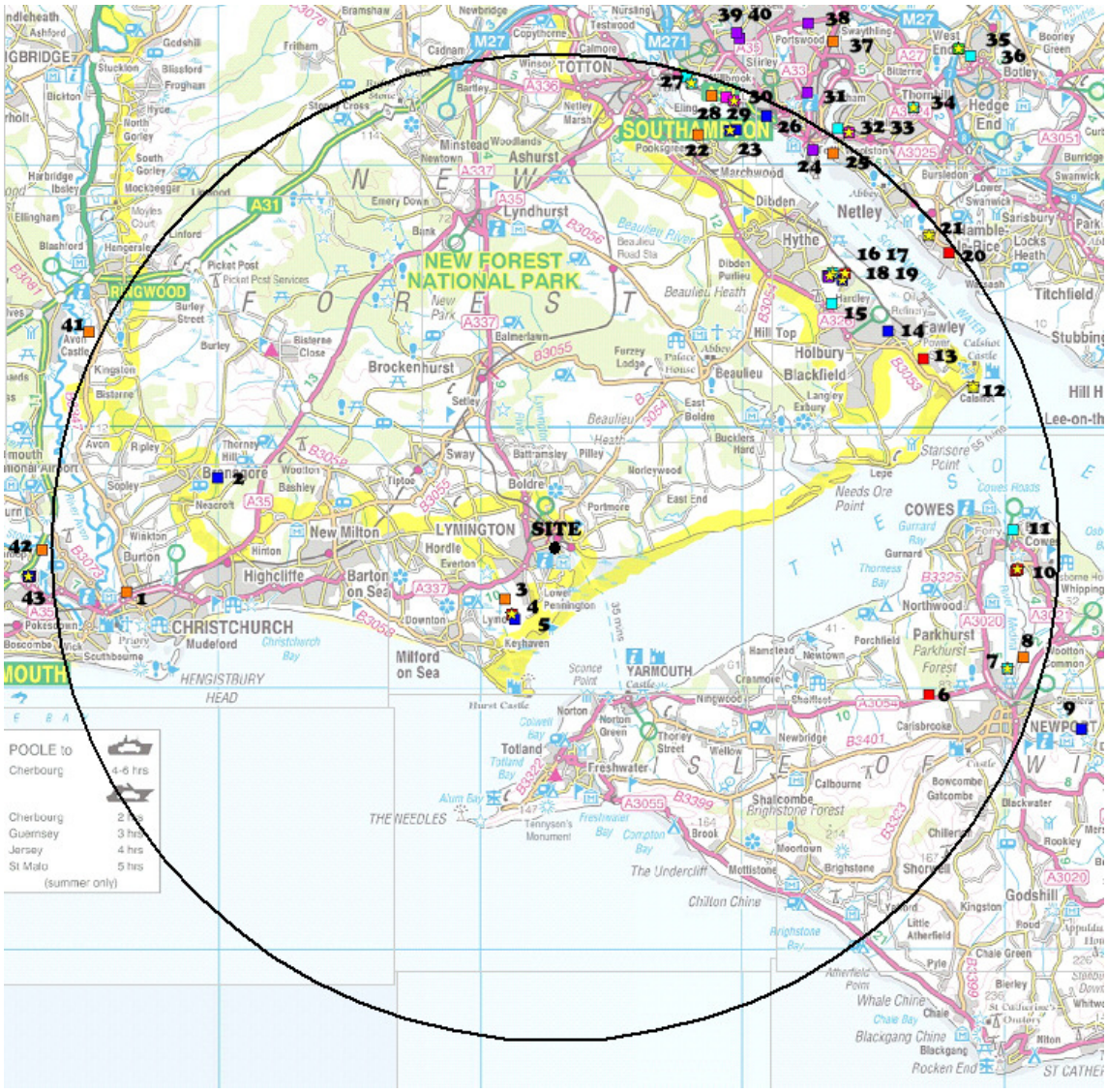


Figure 4-1 Potential sources of industrial pollution around the Lymington site showing 18.5 km radius (see Appendix B.1 for key to map reference numbers)

Industry

There are a number of industrial sites around the Lymington site. Of particular note for this site is the fact that the feeding radius of the breeding black-headed gull extends into the city of Southampton, where industry dominates the waterfront. Indeed, black-headed gulls in breeding plumage can be observed feeding on Southampton Common and in the city centre, confirming that these birds forage in this area during the breeding period (*personal observation*). As the Lymington colonies are the only active breeding colonies of black-headed gulls in this area

today, it is a reasonably safe assumption that, if these birds are breeding, they are likely to be from the Lymington colonies.

Perhaps the most notable industrial site in the area is at Fawley, where the ExxonMobil oil refinery and associated petrochemical complex, and RWE npower oil-fired power station are located side by side. The largest oil refinery in the UK, the Fawley refinery covers approximately 13 km^2, supplies 15% of the UK's oil products and has a capacity of about 16 million tonnes per year, producing over 11 million gallons of petrol, diesel, jet fuel and petrochemical feedstock every day (UKPIA, 2008). Of the refinery output, the majority (about 85%) leaves the refinery by pipeline, 10% by sea and 5% by road or rail (UKPIA, 2008). A chemical manufacturing plant on the site is closely integrated with the refinery, producing a wide range of products for the plastics, synthetic rubber and solvents industries, as well as speciality chemicals, lubricating base oils and additives (UKPIA, 2008). The oil-fired power station was built in the 1960s and historically operated as a 2000MW power station with four 500MW generating units, up until the 1990s (RWE npower, 2004a). Two generating units were subsequently closed during the 1990s and one is now dormant (RWE npower, 2004b). Although the Fawley power station now has the potential to generate 500MW of electricity for the National Grid system, in recent years it has only been used infrequently and is reserved for times of high electricity demand (Dyke *et al.*, 2003; New Forest District Council, 2004b). The station's emissions are dependant largely on the amount of generation at the site (New Forest District Council, 2004b) and although it has only been operated sporadically in recent times, the emissions to air and to Southampton Water would have been much greater in the past when the power station was in constant use. It is likely that the operation of Fawley power station in the past has left a legacy of historical pollution, particularly for heavy metals discharged (see Tables 4-1 and 4-2), which are accumulated in sediments and remain for many years. As previously discussed (Section 4.3), heavy metals may be remobilised into the water column and rendered bioavailable through disturbance of the sediment, for example by dredging activities, strong tides or storm events. Heavy metals may also be accumulated by sediment-dwelling organisms, which are important prey items for many bird species, including black-headed gulls, and may bioaccumulate in the food chain.

The average total annual emissions from industry in the Lymington site area, based on data in Tables 4-1 and 4-2 for the sampling years of 2005 and 2006, are: arsenic: <10 kg to estuary and 63.1 kg to air; cadmium: <3 kg to estuary and 32 kg to air; copper 240 kg to estuary and 92 kg to air; lead: <60 kg to estuary and <500 kg to air; manganese: no reported emissions to estuary and 185 kg to air; nickel: 235 kg to estuary and 3040 kg to air; selenium: no reported emissions

to estuary and 700 kg to air; vanadium: no reported emissions to estuary and 1158 kg to air; zinc: 455 kg to estuary and 1258 kg to air. There are no reported emissions to either estuary or air for cobalt and iron; however, it does not appear that either of these metals is monitored. Note: these calculations for total emissions are based on a 'worst-case scenario' with emissions of less than 'x' being taken as 'x'. Where data are available for both 2005 and 2006, a mean of the two values is taken.

Table 4-1 Metals released to water (kg/year) from industry around the Lymington site (map references refer to Figure 4-1)

Map ref.	Site Name and *Industry Type*	Release Environment	Amount released (kg) and year										
			As	Cd	Co	Cu	Fe	Pb	Mn	Ni	Se	V	Zn
13	Fawley Power Station *Fuel and Power*	Estuary	-	2005: < 1 2006: < 1	-	2005: < 20 2006: < 20	-	2005: < 20 2006: < 20	-	2005: < 20 2006: < 20	-	-	2005: < 100 2006: < 100
14	ExxonMobil Refinery *Fuel and Power, Waste Processes*	Estuary	2005: < 5 2006: < 5	2005: < 1 2006: < 1	-	2005: 220 2006: 180	-	2005: < 20 2006: < 20	-	2005: 100 2006: 290	-	-	2005: < 100 2006: 210
16	Cognis UK Ltd. *Chemical Industry*	Estuary	2005: < 5 2006: < 5	2005: < 1 2006: < 1	-	2005: < 20 2006: < 20	-	2005: < 20 2006: < 20	-	2005: < 20 2006: < 20	-	-	2005: < 100 2006: < 100
29	A & P Southampton *'Other' Industry - Coating, Printing and Textiles*	Estuary	-	-	-	-	-	-	-	-	-	-	2005: < 100

Table 4-2 Metals released to air (kg/year) from industry around the Lymington site (map references refer to Figure 4-1)

Map ref.	Site Name and *Industry Type*	Release Environment	Amount released (kg) and year										
			As	Cd	Co	Cu	Fe	Pb	Mn	Ni	Se	V	Zn
13	Fawley Power Station *Fuel and Power*	Air	2005: 17.7 2006: 17.9	2005: 17.7 2006: 17.9	-	2005: 17.7 2006: 17.9	-	2005: < 100 2006: < 100	2005: < 50 2006: < 10	2005: 283 2006: 237	2005: < 200 2006: < 100	2005: 478 2006: 380	2005: < 100 2006: < 100
14	ExxonMobil Refinery *Fuel and Power; Waste Processes*	Air	2005: 40 2006: 44.6	2005: 10 2006: 14.4	-	2005: 50 2006: 58.5	-	2005: < 100 2006: < 100	2005: 90 2006: 100	2005: 2710 2006: 2810	2005: < 200 2006: < 100	2005: 1002 2006: 1060	2005: 940 2006: 975
23	Veolia Environmental Services Ltd. *Waste Incineration*	Air	2005: < 1 2006: < 1	2005: < 1 2006: < 1	-	2005: < 10 2006: < 10	-	2005: < 100 2006: < 100	2005: < 50 2006: < 50	2005: < 10 2006: < 10	2005: < 200 2006: < 200	2005: < 50 2006: < 50	2005: < 100 2006: < 100
27	Selex Sensors & Airbourne Systems Ltd. *Chemical Industry*	Air	2005: < 1 2006: < 1	-	-	-	-	-	-	-	2005: < 200	-	-
34	Morgan Advanced Ceramics Ltd. *Chemical Industry*	Air	-	-	-	-	-	2005: < 100 2006: < 100	-	-	-	-	-
43	White Rose Environmental Ltd. *Waste Incineration*	Air	2005: < 1 2006: < 1	2005: < 1 2006: < 1	-	2005: < 10 2006: < 10	-	2005: < 100 2006: < 100	2005: < 10 2006: < 10	2005: < 10 2006: < 10	-	2005: < 10 2006: < 10	2005: < 100 2006: < 100

Landfill

Currently there are three landfills actively receiving waste within an 18.5 km radius of the Lymington nesting site. Table 4-3 provides the names and map references (as on Figure 4-1) of active landfill sites within the feeding range of black-headed gulls breeding on the Lymington marshes, and the types of waste they receive (Environment Agency, 2009a).

Table 4-3 Active landfill sites within an 18.5 km radius of the Lymington site (map references refer to Figure 4-1)

Map ref.	Site Name	License Type
2	Holmsley Pit Landfill	Inert Waste
4	Efford Landfill	Household, Commercial and Industrial Waste
9	Lyn Bottom Landfill	Household, Commercial and Industrial Waste

Holmsley Pit Landfill is classified by the Environment Agency as receiving inert waste, such as construction materials, sand and gravel. Efford Landfill, although now closed, was active was during the sampling period of this study (2005-2006); the large site received household, commercial and industrial waste and was situated very near to the sampling site in this study (see Figure 4-1). Lyn Bottom Landfill also receives household, commercial and industrial waste.

Of the active landfill sites in the area, none reported emissions for metals to either air or river/estuary during 2005-2006. However, black-headed gulls frequently forage on landfill sites, and sites containing household, commercial and industrial waste are likely to contain a number of waste items that are potential sources of heavy metals and selenium (see Section 4.3).

Sewage

Several sewage treatment works are located within the area around the Lymington site (Table 4-4). Pennington is the only sewage treatment works (STW) in the area that discharges to the Lymington estuary; Fairlee, Woolston, Milbrook and Portswood STWs all discharge direct to Southampton Water (or, in the case of Fairlee STW on the Isle of Wight, direct to the Solent) and Slowhill Copse discharges to the River Test, which drains into Southampton Water. Slowhill Copse is one of the main outfalls in the area and is a sludge reception site, taking sludge from all New Forest treatment works except Pennington (which goes to Millbrook STW)

and sludge barged across Southampton Water from Woolston and Portswood (New Forest District Council, 2004a). Bournemouth and Christchurch STW discharge directly to Christchurch Bay, and Ringwood STW discharges to Bickerley Mill Stream, a tributary of the River Avon, which subsequently drains into Christchurch Harbour.

In addition to considering the STWs in the local area in terms of the inputs to natural waters as a result of the effluent discharged, it is also important to consider the STWs themselves as a potential source of heavy metal exposure, as black-headed gulls can frequently be found feeding at the sewage works themselves, as well as at sewage outfalls.

Table 4-4 provides data for the major inputs to river/estuary from sewage treatment works in the Lymington site area, using emissions data measured and reported by the Environment Agency (2009a). The average total annual emissions from sewage treatment works in the Lymington site area to river/estuary, for the sampling years of 2005 and 2006, are: arsenic: 99 kg; cadmium: 18 kg; copper: 895 kg; lead: 235 kg to 220 kg; nickel: 267 kg; zinc: 2229 kg. There are no reported emissions for any of the metals of concern in this study to air, and no reported emissions to river/estuary for cobalt, iron, manganese, selenium and vanadium; however, it does not appear that emissions of cobalt and iron are monitored. Note: these calculations for total emissions are based on a 'worst-case scenario' with emissions of less than 'x' being taken as 'x'. Where data are available for both 2005 and 2006, a mean of the two values is taken.

Table 4-4 Metals released to water (kg/year) from sewage treatment works in around the Lymington site (map references refer to Figure 4-1)

Map ref.	Site Name	Release Environment	Amount released (kg) and year										
			As	Cd	Co	Cu	Fe	Pb	Mn	Ni	Se	V	Zn
1	Christchurch STW	Estuary	2002: 21	2002: 9.7	-	2002: 64	-	2002: 33	-	2002: 30	-	-	2002: 340
3	Pennington STW	Estuary	2005: 11 2006: 11.2	2005: <1 2006: <1	-	2005: 80 2006: 80.6	-	2005: 24 2006: 24.2	-	2005: 33 2006: 32.6	-	-	2005: 180 2006: 146
8	Fairlee STW	Estuary	2002: < 6 2007: < 5	2002: < 1 2007: < 1	-	2002: 46 2007: 45.5	-	2002: < 20 2007: < 20	-	2002: < 20 2007: < 20	-	-	2002: 75 2007: < 100
22	Slowhill Copse STW	River	2005: 6.6 2006: 6.71	2005: < 1 2006: < 1	-	2005: 92 2006: 94.6	-	2005: < 20 2006: < 20	-	2005: 40 2006: 30	-	-	2005: 140 2006: 135
25	Woolston STW	Estuary	2005: 6.7 2006: 6.7	2005: < 1 2006: < 1	-	2005: 95 2006: 94	-	2005: < 20 2006: < 20	-	2005: 30 2006: 29.9	-	-	2005: 140 2006: 135
28	Millbrook STW	Estuary	2005: 16 2006: 16.2	2005: < 1 2006: < 1	-	2005: 180 2006: 187	-	2005: 40 2006: 40.2	-	2005: 63 2006: 63.1	-	-	2005: 280 2006: 284
37	Portswood STW	Estuary	2002: 10	2002: < 1	-	2002: 110	-	2002: 33	-	-	-	-	2002: 480
41	Ringwood STW	River	2002: 6.1 2006: < 5	2002: 1.2 2006: < 1	-	2002: 61 2006: 27.2	-	2002: < 20 2006: < 20	-	2002: < 20 2006: < 20	-	-	2002: 73 2006: < 100
42	Bournemouth STW	Estuary	2005: 17 2006: 14.6	2005: 1.3 2006: 1.26	-	2005: 180 2006: 179	-	2005: 29 2006: 20.8	-	2005: 37 2006: 41.7	-	-	2005: 510 2006: 520

4.5.4 Boats and shipping

Commercial shipping

Commercial shipping takes place in the Solent all year round, 24 hours per day. Figure 4-2 shows the major shipping routes in the Solent (Solent Forum, 2005b).

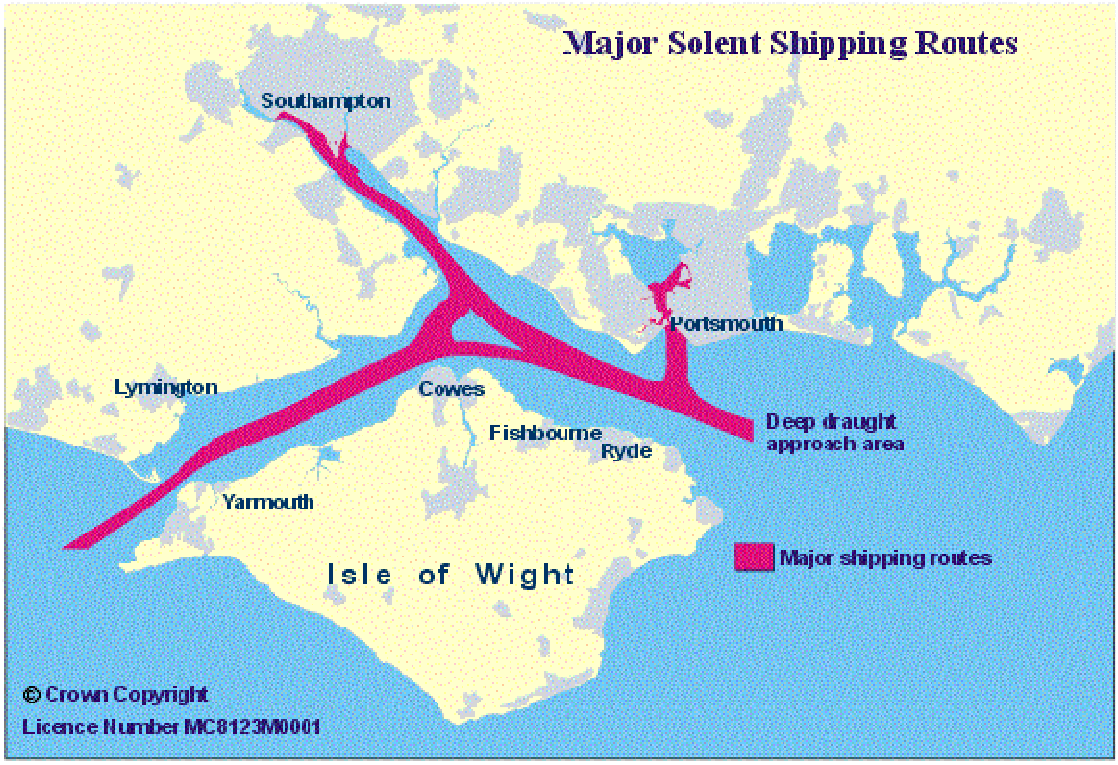


Figure 4-2 Major Solent shipping routes (Solent Forum, 2005b)

Southampton is one of the largest and busiest commercial ports in the country; in excess of 42 million tonnes of cargo is handled annually (ABP, 2007), and Southampton is the UK's number one car import/export port and number two container port (second to Felixstowe; Solent Forum, 2005a). The major port for commercial shipping on the Isle of Wight is Cowes, which handles around 400000 tonnes of cargo annually (Solent Forum, 2005a). The oil terminal for the ExxonMobil refinery at Fawley is the largest independently owned terminal in Europe; it is approximately 1.5 kilometres long and has up to 9 berths, able to accommodate coasters or part-laden tankers up to 350000 tons deadweight and handling some 2000 ship movements each year (UKPIA, 2008). The BP oil terminal at Hamble-le-Rice is used both for the storage and export of crude oil from the Wytch Farm oilfield in tankers up to 110000 tons deadweight and

for the import of products including aviation fuels in tankers up to 20000 tons deadweight, supplying major industry and airports (Mott MacDonald, 2010). Marchwood military port is situated on the western side of Southampton Water and consists of three main jetties, the largest of which is 220 m long and 33 m wide and capable of accepting vessels up to 16000 tonnes (UK Ports, 2010). The facility is used for shipping in support of military administration, training exercises and operations, and for the loading and unloading of approximately 100000 tonnes of military material annually (UK Ports, 2010).

The port of Lymington offers the shortest crossing from the mainland to the Isle of Wight. The Wightlink ferry operating from Lymington carries foot passengers, private cars and a relatively high proportion of commercial vehicles, including around 80% of the tourist coaches that arrive in the Isle of Wight (New Forest District Council, 2004a).

Recreational boating

The Solent is an important area for marine-based recreation, particularly yachting. Much of the recreational boating activity along the New Forest coastline is centred on the Lymington Estuary, and the water-borne recreational pressures within the small river estuary are high, with one craft entering or leaving the river every 30 seconds at the height of the summer season (New Forest District Council, 2004a). Lymington River and Estuary provide moorings for approximately 1600 leisure craft, including 875 at the Lymington and Yacht Haven marinas (New Forest District Council, 2004a). In addition to the permanent moorings the river receives approximately 9000 visiting yachts per annum, and there are on-shore facilities for the storage of approximately 250 craft, and the launching of trailer yachts from two public slipways (New Forest District Council, 2004a). Activities such as boat building and repair and sail making are all carried out in Lymington, which has a long history of coastal trading and shipbuilding (New Forest District Council, 2004a).

Recreational boating is also an important activity around the Beaulieu Estuary, with moorings in much of the Beaulieu River, including a large marina at Buckler's Hard, and between Beaulieu and the estuary. The River Hamble, Hamble Estuary and the area of Southampton Water around the estuary has an international reputation for marine-based recreation, and has moorings for around 1200 vessels for public use, local boat clubs and yards (Crown Estate, 2004). Water-based recreational activities are also carried out in some other areas of Southampton Water and the Solent, with marinas and moorings for recreational craft located along Southampton Water

and on the coast of the Isle of Wight. Cowes Harbour on the Isle of Wight is known as the world's premier yachting centre, hosting numerous yacht races and regattas, including the prestigious annual sailing event, Cowes Week. The Harbour is home to four marinas with extensive mooring facilities, dry storage facilities and visitor pontoons (Cowes Harbour Authority, 2008).

Dredging activities

Maintenance dredging is carried out annually in January-February within the Lymington River and marinas, undertaken by the Harbour Commissioners (New Forest District Council, 2009). Dredging for harbour maintenance at Beaulieu, Hamble and Cowes is only occasional (Solent Protection Society, 2006). Routine maintenance dredging of berths and channels in Southampton Water is also carried out regularly to remove accreting sediment from the Southampton Water system and keep navigational channels open, including dredging at Fawley to maintain access to the oil terminal, amounting to around 100000 m^3/year (Townend, 2008). This regular dredging will lead to remobilisation of metals bound in sediment, rendering them more bioavailable to aquatic organisms (see Section 4.4).

4.6 Poole Harbour

4.6.1 Geology

The geology of Poole Harbour and the surrounding area is similar to that of Lymington, comprising mainly sedimentary rocks, sandstone, gravel and clay, with some chalk (JNCC, 1999). The rocks and soils of the area are unlikely to be a significant source of heavy metals to the environment when compared with anthropogenic sources in the area.

4.6.2 Land use

Urban areas and roads

The area around the Poole site is a mixture of rural and urban areas, with many small villages, few towns and no cities. The north-eastern part of the area (defined in Figure 4-3, below) is extensively urbanised, housing the largest towns in the area - Bournemouth and Poole, with populations of approximately 376000 and 142000, respectively. Of the smaller towns in the area, the market town of Wimbourne Minster (population *ca*. 7000) is also in the north-eastern

part, and the seaside town of Swanage (population *ca*. 11000) and small market towns of Blandford Forum (population *ca*. 3000) and Wareham (population *ca*. 8000) are located to the southeast, northwest and near the centre of the area, respectively. Note: population figures are taken from the Information Britain website (2009).

There are no motorways within the area around the Poole site (in fact, there are no motorways in the whole county of Dorset); however, a network of A-roads runs through the area, including the A31, A35, A338, A348, A350, A3049 and A354, along with a number of other smaller A- and B-roads, and these roads will contribute to the metal pollution of soils, groundwater, rivers and estuaries through road runoff.

There are 39 public car parks in Poole, with eight large car parks located near Poole Quay and the beaches of Sandbanks and Branksome, along with other smaller car parks around the town and several large parking facilities, including some multi-storey, for the town centre shops and shopping centres (Poole Tourism, 2009). As previously mentioned (Section 4.2), car parks create diffuse pollution through increased surface runoff, which has the potential to contaminate the estuary, and such areas are also often exploited by black-headed gulls for scraps of food.

Bournemouth international airport is located just outside the town of Bournemouth; the airport has one runway of around 2 km in length, and handles around one million passengers per year (CAA, 2009). Although no point source emissions are reported from the airport (Environment Agency, 2009a), emissions to air as a result of fuel combustion in plane engines and runoff from the runway are a likely source of heavy metals, and there is potential for minor fuel and oil spills during refuelling operations (see Section 4.2). In addition, the airport generates increased traffic in the area.

Farming and agriculture

The area around the Poole site is largely rural, with a mixture of heathland and woodland alongside arable land and grassland; around 40% of the Green Belt area in the South East Dorset region is currently in agricultural use, amounting to just under 320 km^2 (CPRE & Natural England, 2010). Agriculture in the area is a mixture of livestock farming and arable; although once known for sheep and cattle farming, the pressures faced by livestock farming in recent years mean that much of the farmland has now been converted to arable (Dorset AONB, 2009)

Rivers and riverine inputs

The major rivers draining into Poole Harbour estuary are the Rivers Frome and Piddle (Trent), together with much smaller inputs from the River Sherford, Corfe River and a number of much smaller tributaries (Murdoch & Randall, 2001). However, freshwater contribution to the Harbour is relatively small (Cundy & Croudace, 1995).

The Frome rises in West Dorset and flows east towards the town of Dorchester and on to Poole Harbour; it is joined by a number of tributaries including the Cerne, Sydling Water and Hooke Stream (Langston *et al.*, 2003). The Piddle rises near the village of Buckland Newton and flows south-east towards Poole Harbour; major tributaries include the Devil's Brook and the Bere Stream (Langston *et al.*, 2003). The Corfe and Sherford Rivers have a much smaller input to the Harbour, and their catchments are largely rural. As the catchment of the rivers entering Poole Harbour is mainly rural, the rivers and their tributaries are impacted by runoff from agricultural land, which may lead to pollution from pesticide residues, fertilisers and farm animal waste (Drake, 2007). In addition, urban developments create diffuse pollution through increased surface runoff, and have potential to contaminate watercourses with heavy metals (Comber & Gunn, 1996). Within the catchment of the rivers that enter Poole Harbour the main urban influences are the towns of Dorchester and Wareham, and the town of Poole and suburbs of Bournemouth also provide potentially more direct sources of urban and industrial contamination to Poole Harbour and Poole Bay.

The River Stour and River Allen (a tributary of the Stour) are also located within the area (defined in Figure 4-3, below), and may therefore be used as feeding sites by black-headed gulls breeding at Poole. The Stour rises in Stourhead, an estate near the small town of Mere in Wiltshire, from where it flows south into Dorset. At Wimbourne Minster, the Stour and the Allen join together and subsequently flow southeast, ultimately draining into Christchurch Estuary. The catchments of the Stour and the Allen are rural, with both rivers flowing through agricultural land and small countryside towns and villages.

4.6.3 Industry and waste

Figure 4-3 shows the potential sources of industrial pollution within an 18.5 km radius of the Poole site (shown by the black circle; Environment Agency, 2009a). A full list of the sites marked on this map is provided in Appendix B, Table B.2. For the purpose of this review, only those sites reported to release the metals of concern in this study to air or natural waters will be discussed in detail.

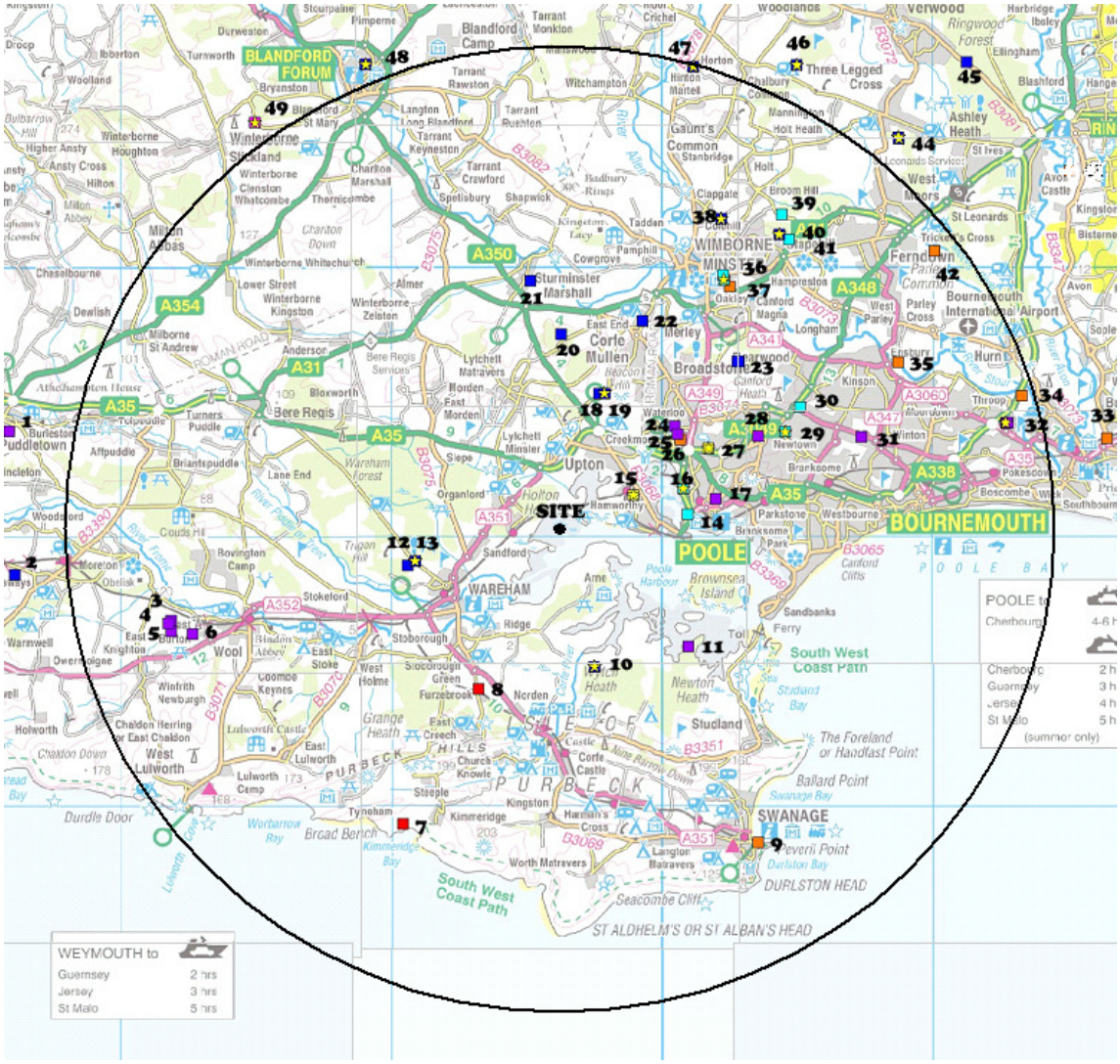


Figure 4-3 Potential sources of industrial pollution around the Poole site showing 18.5 km radius (see Appendix B.2 for key to map reference numbers)

Industry

Historical inputs into the Harbour waters from chemical industries in Poole have meant that pollution studies in the area have focused heavily on metals. Metal concentrations are particularly high in the sediment, waters and biota of the Holes Bay area, where decades of toxic metal discharges have occurred. In the 1970s, dissolved metal concentrations in Holes Bay often exceeded values considered typical for the English Channel by 30-40 fold for copper, nickel and zinc (average concentrations: copper 18 µg/l, nickel 8 µg/l, zinc 68 µg/l), and more than 100 fold for cadmium and lead (cadmium 7.4 µg/l, lead 2.5 µg/l; Boyden, 1975). The majority of trade discharges to Holes Bay have now ceased and the above data for dissolved

concentrations are over 30 years old, indicating historic concentration of metals in water. However, sediments have accumulated these metals and, as a result, metals in sediments in Poole Harbour and particularly in Holes Bay in recent studies still exceeded sediment quality guidelines widely (Langston *et al.*, 2003). The legacy of historic pollution will remain in the Harbour for many years as metals do not break down in the environment and the enclosed nature and poor flushing of Poole Harbour means that the distribution of contaminated sediment is limited. Metals can accumulate in the organisms that live within the sediment and may be bioaccumulated up the food chain, and species such as worms and clams, again particularly in Holes Bay, have demonstrated significant bioaccumulation of a number of metals, including cadmium, copper, zinc and selenium, above normal concentrations (Langston *et al.*, 2003).

Today, the Harbour still supports many industries of differing scales, such as boat building, factories and sail lofts (Drake, 2007). One significant industry located in the south of the Harbour is the onshore oilfield, Wytch Farm. Wytch Farm is Europe's largest onshore oilfield, with extended reach drilling techniques used to exploit oil deposits under Poole Bay, which are then distributed from the Harbour via underground pipes to the BP oil terminal in Hamble-le-Rice on Southampton Water (Drake, 2007). Production in 2006 stood at between 20-30000 barrels per day; however, there are no effluent outputs from the operation of Wytch Farm as discharges are collected and returned to the oil-bearing strata to aid extraction, and Wytch Farm has won numerous awards for environmental achievement (IPIECA, 2003).

In terms of reported emissions from industry in the area around the Poole site to natural waters, the only reported emissions during the study period are for cadmium, with an average <1 kg cadmium per annum being released to the River Frome (Table 4-5), which drains into Poole Harbour. The average total annual emissions to air from industry in the area around the Poole site, based on data in Table 4-6 for the sampling years of 2005 and 2006, are: arsenic: <1 kg; cadmium: <3 kg; copper: <10 kg; lead: <100 kg; manganese: <10 kg; nickel: <30 kg; vanadium: <10 kg; zinc: <200 kg. There are no reported emissions to air for cobalt, iron and selenium; however, it does not appear that emissions of cobalt or iron are monitored.

Table 4-5 Metals released to water (kg/year) from industry around the Poole site (map references refer to Figure 4-3)

Map ref.	Site Name and *Industry Type*	Release Environment	Amount released (kg) and year										
			As	Cd	Co	Cu	Fe	Pb	Mn	Ni	Se	V	Zn
49	Faccenda Group *Animal, Vegetable and Food Industry*	River	-	2005: < 1 2006: < 1	-		-	-	-	-	-	-	-

Table 4-6 Metals released to air (kg/year) from industry around the Poole site (map references refer to Figure 4-3)

Map ref.	Site Name and *Industry Type*	Release Environment	Amount released (kg) and year										
			As	Cd	Co	Cu	Fe	Pb	Mn	Ni	Se	V	Zn
15	Poole Technical Plating Services *Metal Production and Processing*	Air	-	-	-	-	-	-	-	2005: < 10 2006: < 10	-	-	-
29	Sigma-Aldrich, Inorganic Chemicals *Chemical Industry*	Air	-	2005: < 1	-	-	-	-	-	-	-	-	2005: < 100
32	White Rose Environmental *Waste Incineration*	Air	2006: < 1	2006: < 1	-	2006: < 10	-	2006: < 100	2006: < 10	2006: < 10	-	2006: < 10	2006: < 100
36	Flight Refuelling, Inorganic Chemicals *Chemical Industry*	Air	-	-	-	-	-	-	-	2005: < 10 2006: < 10	-	-	-
41	Portsmouth Aviation, Inorganic Chemicals *Chemical Industry*	Air	-	2005: < 1 2006: < 1	-	-	-	-	-	-	-	-	-

Landfill

There are a number of active landfill sites around the Poole site; Table 4-7 provides the names and map references (as on Figure 4-3) of active landfill sites within the feeding range of black-headed gulls breeding on the Poole marshes, and the types of waste they receive (Environment Agency, 2009a).

Table 4-7 Active landfill sites within an 18.5 km radius of the Poole site (map references refer to Figure 4-3)

Map ref.	Site Name	License Type
2	Warmwell Landfill	Inert Waste
12/13	Tatchells Landfill	Household, Commercial and Industrial Waste
20	Henbury Pit Landfill	Inert Waste
22	Beacon Hill Landfill	Household, Commercial and Industrial Waste
23	White's Pit Landfill	Household, Commercial and Industrial Waste
38	Pound Bottom Landfill	Household, Commercial and Industrial Waste
40	Squabb Wood Landfill	Household, Commercial and Industrial Waste
44	Homefield Pit Landfill	Inert Waste
45/47	Somerley Landfill	Household, Commercial and Industrial Waste
46	Hemitage Farm Landfill	Household, Commercial and Industrial Waste

Only one landfill reports emissions direct to natural waters; all the other landfills reported emissions to the sewer system (Environment Agency, 2009a) and are therefore treated before discharge via sewage treatment works. However, black-headed gulls frequently forage on landfill sites, and sites containing household, commercial and industrial waste, thus seven of the sites above in Table 4-7 are likely to contain a number of waste items that are potential sources of heavy metals and selenium (see Section 4.3). The other three landfills in the area -Warmwell, Henbury Pit and Homefield Pit - are classified by the Environment Agency as receiving inert waste, which may include metal, wood, bricks, asphalt or cement concrete, and other building construction materials such as plaster, drywall, siding, shingles, insulation, and glass, which may also be significant sources of metals (see Section 4.3).

Table 4-8 provides data for the inputs to river/estuary from landfill in the Poole site area, using emissions data measured and reported by the Environment Agency (2009a). The average annual

emissions to natural waters from landfill in the Poole site area come only from the Henbury Pit landfill, with emissions of cadmium (<1 kg), copper and lead (both <20 kg) reported in 2002. Unfortunately, no data were available for emissions during 2005-2006, the period of this study. Henbury Pit landfill releases to the River Stour, which in turn drains into Christchurch Harbour. Although the emissions from this landfill will not ultimately reach Poole Harbour, the gulls breeding on the Poole marshes may well feed in and around parts of the River Stour, as well as foraging on the landfill site itself. There are no reported emissions for any of the metals of concern in this study to air, and no reported emissions to natural waters for cobalt, iron, manganese, selenium and vanadium; however, it does not appear that emissions of cobalt or iron are monitored.

Sewage

Poole Harbour directly receives treated discharges from Poole STW, which is perhaps one of the most significant and sensitive discharges in the area, particularly because of the enclosed and sheltered nature of the receiving environment in the northeast corner of Holes Bay (Langston *et al.*, 2003). The STWs at Bournemouth (both Kinson and Holdenhurst), Wimbourne and Palmersford all discharge to the River Stour, which ultimately drains into Christchurch Harbour, where the Christchurch STW discharges directly. Although all these STWs and the rivers they discharge to are within (or in close proximity of) the feeding radius of the black-headed gulls nesting at Poole, the ultimate receiving area of Christchurch Harbour is outside of this radius and is an area unlikely to be exploited by the Poole gulls during breeding. However, the gulls may well feed in and around the rivers to which treated wastewater is discharged. Swanage STW discharges directly into the English Channel, approximately 400m from the shore, via an existing Victorian pipeline (watertreatment.com, 2006). As previously mentioned, black-headed gulls can frequently be found feeding at the sewage works themselves, and it is important to consider the STWs themselves, as well as the outfalls and discharges to natural waters, as a potential source of heavy metal exposure.

Table 4-9 provides data for the major inputs to river, estuary and sea from STWs within the feeding radius of a breeding black-headed gull nesting on the Poole marshes, using emissions data measured and reported by the Environment Agency (2009a). The average annual emissions from sewage treatment works entering Poole Harbour are those from Poole STW, amounting to: 14.8 kg arsenic; 1.19 kg cadmium; 153 kg copper; 42.1 kg lead; 308 kg nickel; and 334.5 kg zinc. There are no reported emissions to air from any of the STWs in Table 4-9 for any of the metals of concern in this study, and no reported emissions to river, sea or estuary for cobalt,

iron, manganese, selenium and vanadium; however, it does not appear that emissions of cobalt or iron are monitored. Note: where data are available for both 2005 and 2006, a mean of the two values is taken.

Table 4-8 Metals released (kg/year) from landfill around the Poole site (map references refer to Figure 4-3)

Map ref.	Site Name	Release Environment	Amount released (kg) and year										
			As	Cd	Co	Cu	Fe	Pb	Mn	Ni	Se	V	Zn
18	Henbury Pit Landfill	River	-	2002: < 1	-	2002: < 20	-	2002: < 20	-	-	-	-	-

Table 4-9 Metals released to water (kg/year) from sewage treatment works around the Poole site (map references refer to Figure 4-3)

Map ref.	Site Name	Release Environment	Amount released (kg) and year										
			As	Cd	Co	Cu	Fe	Pb	Mn	Ni	Se	V	Zn
9	Swanage STW	Sea	2005: < 5 2006: < 5	2005: < 1 2006: < 1	-	2005: < 20 2006: < 20	-	2005: < 20 2006: < 20	-	2005: < 20 2006: < 20	-	-	2005: < 100 2006: < 100
26	Poole STW	Estuary	2005: 15 2006: 14.6	2005: 1.2 2006: 1.18	-	2005: 180 2006: 126	-	2005: 42 2006: 42.2	-	2005: 330 2006: 286	-	-	2005: 330 2006: 339
33	Christchurch STW	Estuary	2002: 21	2002: 9.7	-	2002: 64	-	2002: 33	-	2002: 28	-	-	2002: 340
34	Bournemouth (Holdenhurst) STW	River	2005: 17 2006: 14.6	2005: 1.3 2006: 1.26	-	2005: 180 2006: 179	-	2005: 29 2006: 20.8	-	2005: 37 2006: 41.7	-	-	2005: 510 2006: 520
35	Bournemouth (Kinson) STW	River	2002: 10	2002: 2.1	-	2002: 97	-	2002: 23	-	2002: 21	-	-	2002: 100
37	Wimborne STW	River	2005: < 5 2006: < 5	2005: < 1 2006: < 1	-	2005: 29 2006: 28.9	-	2005: < 20 2006: < 20	-	2005: < 20 2006: < 20	-	-	2005: < 100 2006: < 100
42	Palmersford STW	River	2002: 16	2002: 35	-	2002: 72	-	2002: 170	-	2002: 64	-	-	2002: 310

4.6.4 Boats and shipping

Recreational boating

Poole Harbour is renowned for its yachting, with eight clubs providing over 7500 members with racing and cruising activities all year round, in addition to around 5000 yachts visiting each year (PHC, 2007). There are seven main marinas in Poole Harbour, with approximately 2500 swinging moorings within the Harbour, 2300 sheltered marine and pontoon berths and a further 100+ moorings occupied by the Environment Agency, in addition to dry boat storage facilities with a capacity for approximately 2000 boats to be stored (Drake, 2007). The largest marina in Poole Harbour is Holes Bay, with berths for around 800 craft (Drake, 2007).

Commercial shipping

Approximately 0.2 km^2 of Poole Harbour are devoted to commercial port operations (Langston *et al.*, 2003) with a variety of different cargoes are handled and stored, including bulk cargo imports of steel, timber, bricks, fertiliser, grain, aggregates and palletised traffic, and exports including clay, sand, fragmented steel and grain (Drake, 2007). Regular ferry services operate between Poole and Cherbourg, St. Malo and the Channel Islands, with both passenger and freight services and a chain ferry operates a regular, year-round car and foot passenger ferry service across the Harbour to the Studland peninsular (PHC, 2007). Sightseeing vessels also operate from Poole Harbour to Brownsea and other islands around the Harbour during tourist season (PHC, 2007).

Dredging activities

In a busy commercial port such as Poole Harbour, maintenance dredging is necessary to keep navigational channels open, and is carried out routinely by Poole Harbour Commissioners and by third party operators to maintain access (Drake, 2007). Maintenance dredging in Poole Harbour amounts to an average displacement of 70000m^3 sediment per year, with additional smaller quantities dredged for access to marinas (Langston *et al.*, 2003). As previously mentioned (Section 4.4), regular dredging will lead to remobilisation of metals bound in sediment, rendering them more bioavailable to aquatic organisms.

4.7 Raby Estate

4.7.1 Geology

The deep strata of the North Pennines are slates and volcanic rocks, comprising a large granite body and repeating layers of limestone, shale and sandstone (North Pennines AONB Partnership, 2010). Owing to the large body of granite underlying the area, lead and zinc have been mined in the past (see Section 4.6.3 'Industry'), and there may be a significant contribution of arsenic, copper, lead, nickel, vanadium and zinc from natural weathering of rocks and soils in the area of the Raby site.

4.7.2 Land use

Urban areas and roads

The area surrounding the Raby site is one of the most remote nature reserves in England; as a result, there is very little in the way of domestic areas in the vicinity. In fact, within the feeding area of a breeding black-headed gull (as shown in Figure 4-4 below, taking 18.5 km as the foraging radius) around the Raby site there is only one small town, Appleby-in-Westmorland, which has a population of less than 3000 (Eden District Council, 2008). The village of Brough also lies within the area, with a population of around 800 (Eden District Council, 2008).

There are no roads directly linked to the Raby site. The closest main road to the site is the B6277, from which a smaller, unnamed road leads to the Cow Green reservoir and the car park located there (see Figure 4-4, below), which is the only car park in the area (English Nature, 2004). The nearest point of the B6277 to the site is approximately 4 km northeast, and the unnamed road leading to Cow Green reservoir is approximately 2 km north. While both of these roads run at a ground height sufficient that runoff from the road could potentially reach the Cow Green reservoir, there is likely to be very little traffic on these roads as they lead only to and from the reservoir, and pollution from vehicles is therefore likely to be low. The other roads in the area (the A66, A689, B6276 and various smaller roads) are all located on land lower than the reservoir and runoff water from these roads will not therefore impact on the reservoir directly. However, all of the roads mentioned here are within the feeding radius of breeding black-headed gulls, and the gulls may therefore be impacted by runoff and pollution arising as a result of the roads, through foraging in the areas surrounding them that have been contaminated

as a result of road runoff, and also via atmospheric pollution arising from vehicle emissions and resultant wet and dry deposition.

Farming and agriculture

The land around the Raby site is predominantly heather moorland and the only type of farming carried out in the area is traditional upland farming with land managed by grazing sheep. The land is poor in terms of agriculture, owing to poor soils and climate for growth of crops, and is classified by English Nature's Agricultural Land Classification as Grade 4/5, Poor/Very Poor, and as a 'Less Favoured Area' (Durham County Council, 2004). Runoff and contamination from agricultural practices is therefore not an issue of concern in this area.

Military firing range

Parts of the Ministry of Defence Warcop military training area and firing range are located to the south of the Raby site, in parts of Mickel and Cronkley Fells. Military firing ranges have been shown to give rise to metal pollution as a result of the use of incendiary devices and spent bullets (NC DPPEA, 1998). Lead is the primary contaminant of concern at military ranges, in addition to copper and zinc, which are the primary components in shell casings and jackets and, to a lesser extent, nickel and arsenic (NC DPPEA, 1998). On average, new bullets and pellets consist of over 90% lead, less than 2% arsenic and less than 0.5% nickel (Robinson *et al.*, 2008). Tracer and incendiary bullets also contain zinc (Robinson *et al.*, 2008). Once in the soil, bullets and bullet fragments gradually oxidise through weathering by air and water, organic acids and microbial activity (Lin *et al.*, 1995; Johnson *et al.*, 2005). The most important factor governing the rate of bullet oxidation is the pH of the soil, with lower pH soils leading to greater oxidation rates than those of higher pH (Labare *et al.*, 2004). The Warcop military training area comprises acid heath and grassland, mixed with some calcareous grassland (Turner *et al.*, 2003), thus the soils in the area will be mainly acidic, and the rate of bullet oxidation will be reasonably rapid. Indeed, it has been demonstrated with field experiments that concentrations of lead, copper and nickel are significantly elevated in soil samples taken from a military firing range (NC DPPEA, 1998; Robinson *et al.*, 2008). Robinson *et al.* (2008) found lead, copper and nickel at mean concentrations of 10171 mg/kg, 4125 mg/kg and 917 mg/kg, respectively; in the case of lead the highest concentration measured in the firing range soils in this study was 160 times higher than the soil quality standard (over 85000 mg/kg, compared to a quality standard of 530 mg/kg). Elevated concentrations of lead in invertebrates on firing ranges has also been reported (Migliorini *et al.*, 2003).

Although metals released as a result of bullet oxidation will mainly remain in the soil, they may migrate with surface runoff and erosion (Craig *et al.*, 1999). In addition, soil-dwelling organisms will accumulate heavy metals from contaminated soils, and these metals can then be bioaccumulated by higher organisms through the diet, for example by black-headed gulls feeding on earthworms.

Rivers and riverine inputs

The black-headed gulls breeding at the Raby site nest near Cow Green Reservoir, a two-mile long reservoir built in the late 1960s. The River Tees rises at Cross Fell and flows southeast to feed into Cow Green Reservoir, before flowing further eastwards through the small market town of Middleton-in-Teesdale (population *ca.* 1500; Teesdale.co.uk, 2009) to eventually drain into the North Sea between Hartlepool and Redcar, on the northeast coast of England. The River Balder, a small tributary of the River Tees, flows into the Balderhead Reservoir which in turn feeds the Blackston Reservoir and subsequently the Hury Reservoir, all of which are located to the southeast of the Raby site. Two other reservoirs are located just north of these - Selset Reservoir and Grassholme Reservoir - both of which control the flow of the River Lune, another small tributary of the River Tees. The area of the Tees catchment relevant to the site of interest in this study is entirely rural and largely unpopulated, with the river flowing through unmanaged heathland, acid and calcareous grassland and blanket bog.

4.7.3 Industry and waste

Figure 4-4 shows the potential sources of industrial pollution within an 18.5 km radius of the Raby site (shown by the black circle; Environment Agency, 2009a). A full list of the sites marked on this map is provided in Appendix B, Table B.3. For the purpose of this review, only those sites reported to release the metals of concern in this study to air or natural waters will be discussed in detail.

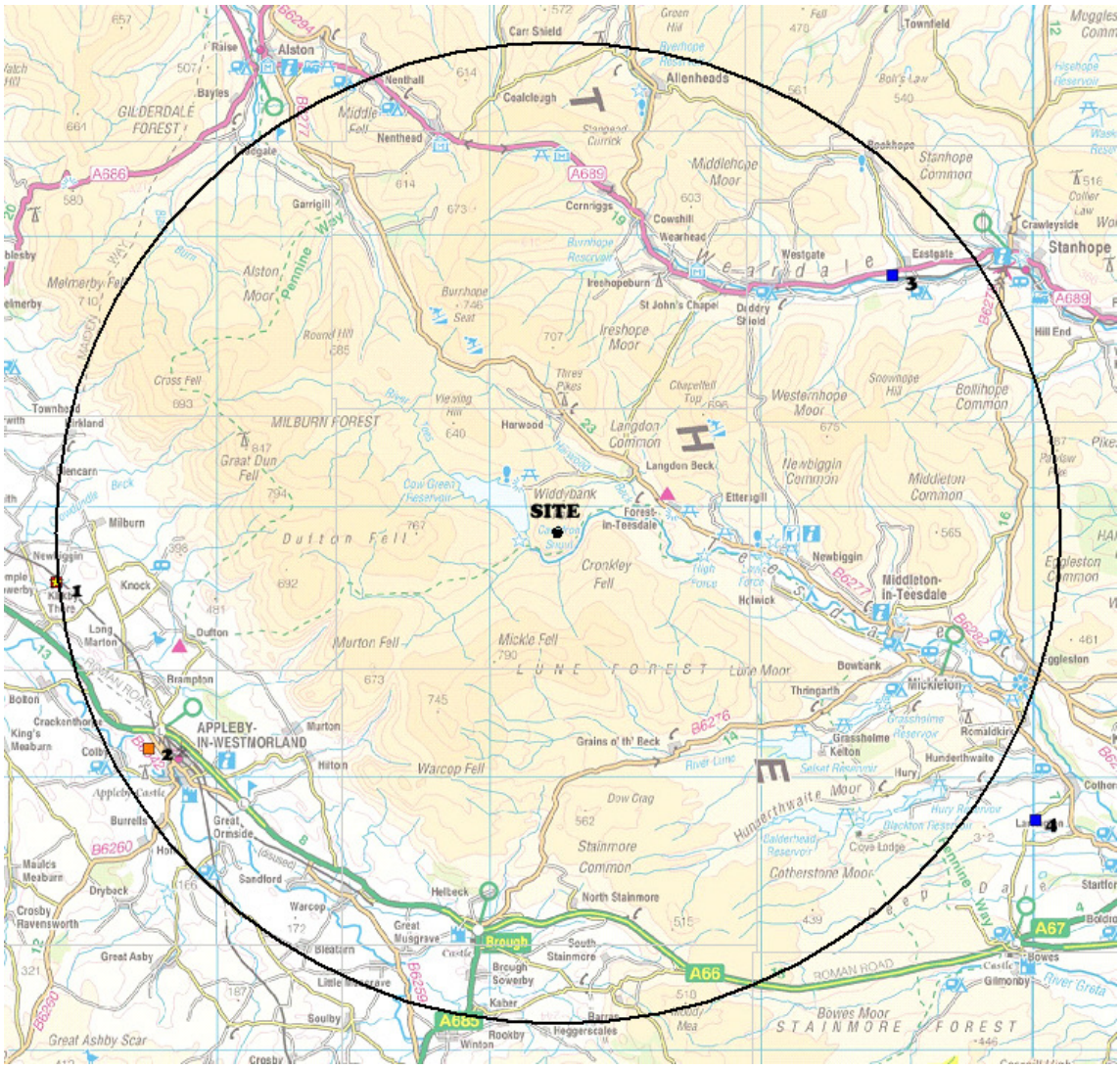


Figure 4-4 Potential sources of industrial pollution around the Raby site showing 18.5 km radius (see Appendix B.3 for key to map reference numbers)

Industry

There is no active industry reporting releases of metals to either water or air within an 18.5 km radius of the Raby site. Although the British Gypsum processing plant (map ref. 1), which is involved in the production and processing of plaster and plasterboard, is active and was operating during the sampling period, there were no reported emissions of metals to the environment (Environment Agency, 2009a).

Although there is no current active industry in the Raby site area, the area has a long history of mining activity. Discharges of metals were at their greatest during the peak period of active

mining, however, significant inputs of dissolved and particulate metals still occur through erosion of soil heaps and tailings deposited adjacent to water channels, and past discharges have left a substantial reservoir of contaminated sediments which can be remobilised during flood events (Environment Agency, 2008). In the North Pennines, a hydraulic form of mining known as 'hushing' was carried out, involving the construction of dams upslope of mineral veins, then opening sluices to release water, with the resulting torrent exposing underlying bedrock and mineral veins, which were worked using simple quarrying techniques (Environment Agency, 2008). Hushing was repeated periodically to remove debris from the workings. All of these methods involved water, and led to the transfer of significant quantities of metal-rich, fine grained sediment to river systems (Environment Agency, 2008). At abandoned mines, such as the lead mines at the Raby site, wastes from ore processing were commonly deposited next to or in rivers, and lead-, zinc-, cadmium- and copper-bearing sulphides and carbonates occur in ore bodies and mine waste-tips in the Tees Basin (Dunham, 1948; Ixer *et al.*, 1979; Vaughan & Ixer, 1980; Young *et al.*, 1985; Dunham, 1990). The river catchments of the Northern Pennines, particularly the upper Tees (Hudson-Edwards *et al.*, 1997), which drains into Cow Green reservoir alongside which the Raby black-headed gull colony breeds, are among the most affected by mining-related metal contamination in England and Wales, with total metal outputs to the river system from mining activities amounting to 4064000 tonnes of lead and 271000 tonnes of zinc (Manning, 1959; Schnellman & Scott, 1970; Lewin & Macklin, 1987). Studies have shown that sediment metal concentrations on the Tees catchment are highest in the areas where mining was carried out in the past (up to 6880 mg/kg lead, 1920 mg/kg zinc and 5.95 mg/kg cadmium), such as Raby, and show an overall decrease downstream of mining areas (Hudson-Edwards *et al.*, 1997). These sediment concentrations are up to ten times greater than the baseline values defined for the Tees pre-mining (Lee, 1989) and for median values determined for upstream sediment from the British Geological Survey's Geochemical Baseline Survey of the Environment (G-BASE; British Geological Survey, unpublished data).

In natural waters a large proportion of metals is associated with sediment (Hudson-Edwards *et al.*, 1997), and whilst river channels can be regarded as temporary stores of metal-rich sediments, floodplains, wetlands, reservoirs, lakes and estuaries are the long-term sinks for metal storage in river basins and large quantities of metal contaminants can be stored in sediments of these environments. As previously mentioned (Section 4.3), metals in sediments can be remobilised and made bioavailable (Lewin *et al.*, 1977; Macklin, 1985; Macklin *et al.*, 1992; Hudson-Edwards *et al.*, 1999; Brewer *et al.*, 2005; Cave *et al.*, 2005). These highly contaminated materials can be mobilised during disruption of the sediment and weathering of

contaminated soils, alluvium and mining wastes, for example during floods and periods of high river flow (Environment Agency, 2008), during which a large total surface area of metal-contaminated sediment is brought into contact with water, causing desorption of metals from sediment surfaces to the aqueous phase (Nagorski *et al.*, 2003; Neal & Davies, 2003; Gozzard *et al.*, 2006), rendering them bioavailable to organisms living and feeding in the river. Conditions of low pH (below pH 5) can accelerate weathering processes and cause dissolution of metal-bearing minerals and release of their metals to the solute phase. The soils and stream, river and reservoir waters and sediments of the area around the Raby site vary in pH from blanket peat bogs and streams and rivers fed by acidic drainage from them (pH *ca.* 4.0) to calcareous springs and associated soils and streams and rivers fed by alkaline drainage (pH *ca.* 8.3; Turner *et al.*, 2003), and thus the rate of weathering will be dependant on the specific area and the sources feeding the rivers and streams.

Landfill

There is one landfill site within an 18.5 km radius of the Raby site, and one further landfill site just outside of this radius (map references 1 and 4, Figure 4-4). Details of the types of waste received by landfills in the Raby area are provided in Table 4-10.

Table 4-10 Active landfill sites within an 18.5 km radius of the Raby site (map references refer to Figure 4-4)

Map ref.	Site Name	Type of waste received
1	Kirkby Thore Landfill	Gypsum waste from plasterboard manufacture
4	Cotherstone Moor Landfill	Sludge disposal

The Kirkby Thore landfill was operated by British Gypsum, and although it has subsequently closed, the landfill site was in operation during the period of sampling in this study, and afterwards, until closure in June 2007 (British Gypsum, 2009a). Kirkby Thore landfill received waste from the plaster and plasterboard manufacture carried out at the Kirkby Thore industrial site (see previous section 'Industry'); however, owing to the fact that 100% of gypsum produced as a result of the industrial site is now recycled, there is no longer a requirement for landfill (British Gypsum, 2009b). Gypsum is a soft mineral composed of calcium sulphate dihydrate ($CaSO_4.2H_2O$) and a by-product of the production of plaster and plasterboard (Euro Gypsum,

2007); there is no metal content and no metals seem to be associated with gypsum. However, some emissions of metals to the river environment have been documented as a result of waste processes carried out at Kirkby Thore landfill (Environment Agency, 2009a), and are detailed in Table 4-11. No emissions to air were reported from this landfill.

The landfill at Cotherstone Moor is a water treatment sludge disposal area operated by Northumbrian Water Ltd., receiving sludge pumped from Lartington Sewage Treatment Works (Environment Agency, 2009a). Emissions of metals from the Cotherstone Moor landfill have been reported to both air and the river environment (Environment Agency, 2009a). Data for these emissions are provided in Table 4-11. Data for this landfill relate to 2004; unfortunately, emissions data were not available for 2005 or 2006.

The average total annual emissions from landfill in the Raby site area, based on data in Table 4-11 for the sampling years of 2005 and 2006, are: arsenic: <10 kg to river and <1 kg to air; cadmium: <1 kg to river and <1 kg to air; copper <40 kg to river and <1 kg to air; lead: <40 kg to river and <100 kg to air; manganese: no reported emissions to river and <50 kg to air; nickel: <40 kg to river and <10 kg to air; zinc: <200 kg to river and <100 kg to air. There are no reported emissions to either river or air for cobalt, iron, selenium and vanadium; however, it does not appear that emissions of cobalt or iron are monitored.

Sewage

There is one sewage treatment works located within the area surrounding the Raby site: the Appleby sewage treatment works, operated by United Utilities Water plc. Emissions data for this site (Environment Agency, 2009a) are provided in Table 4-12. The average annual emissions to the river environment from sewage treatment works in the area of the Raby site, during the 2005-2006 period, are: arsenic <5 kg; cadmium <1 kg: copper <200 kg: lead: <20 kg; nickel <20 kg; and zinc: <100 kg. No emissions for cobalt, iron, manganese or nickel were reported from sewage treatment works to water, and no emissions of any metal were reported to air.

Table 4-11 Metals released (kg/year) from landfill sites the Raby site (map references refer to Figure 4-4)

Map ref.	Site Name	Release Environment	Amount released (kg) and year										
			As	Cd	Co	Cu	Fe	Pb	Mn	Ni	Se	V	Zn
1	Kirkby Thore, Waste Landfilling	River	2005: < 5 2006: < 5	-	-	2005: < 20 2006: < 20	-	2005: < 20 2006: < 20	-	2005: < 20 2006: < 20	-	-	2005: < 100 2006: < 100
4	Cotherstone Moor, Waste Landfilling	River	2004: < 5	2004: < 1	-	2004: < 20	-	2004: < 20	-	2004: < 20	-	-	2004: < 100
4	Cotherstone Moor, Waste Landfilling	Air	2004: < 1	2004: < 1	-	2004: < 1	-	2004: < 100	2004: < 50	2004: < 10	-	-	2004: < 100

Table 4-12 Metals released (kg/year) from sewage treatment works around the Raby site (map references refer to Figure 4-4)

Map ref.	Site Name	Release Environment	Amount released (kg) and year										
			As	Cd	Co	Cu	Fe	Pb	Mn	Ni	Se	V	Zn
2	Appleby Sewage Treatment Works	River	2005: < 5 2006: ND	2005: < 1 2006: < 1	-	2005: < 20 2006: < 20	-	2005: < 20 2006: < 20	-	2005: < 20 2006: ND	-	-	2005: < 100 2006: < 100

ND = no data

4.8 Summary of sources

The following section provides a summary of the point-source inputs to air and natural waters from industry and waste processes, and the key non-point (diffuse) sources in the area of each of the sampling sites, based on the information provided in this chapter.

4.8.1 Point sources

Table 4-13 provides a summary of the average total discharges of metals from industrial sites, sewage treatment works and landfills within the feeding range of breeding black-headed gulls for each of the sampling areas, from Environment Agency data (2009a) provided in Sections 4.5.3, 4.6.3 and 4.7.3. No data were available regarding discharges of cobalt and iron.

Table 4-13 Point source discharges of metals (kg/year) from industry and waste to natural waters and air around the Lymington, Poole and Raby sites, average for 2005 and 2006

	LYMINGTON		POOLE		RABY	
	WATER	AIR	WATER	AIR	WATER	AIR
As	109	63	15	1	15	1
Cd	21	32	3	3	2	1
Cu	1135	90	173	10	240	1
Pb	295	500	62	100	60	100
Mn	0	185	0	10	0	50
Ni	502	3040	308	30	60	10
Se	0	700	0	0	0	0
V	0	1158	0	10	0	0
Zn	2684	1258	335	200	300	100

The data in Table 4-13 show that, in terms of point discharges to the environment monitored and consented by the Environment Agency, the releases to both air and natural waters during the sampling period (2005-2006) were higher in the area around the Lymington site than either the Poole or Raby areas for all metals. Point-source discharges to natural waters and air around the Poole site for the period of this study were of a similar order to those around the Raby site for arsenic, cadmium and lead; no discharges of selenium were reported for either site. For nickel and zinc, releases to both air and water were higher around the Poole site than the Raby site.

Overall, discharges of copper and manganese to the environment were higher for the Raby area than the Poole area. For vanadium, no discharges were reported to either air or water around the Raby site, and only a small amount (10 kg/year average) of vanadium was discharged to air in the area around the Poole site.

4.8.2 Non-point sources

Diffuse pollution is now more evident since the quality of point source discharges has been improved and is carefully monitored and controlled (CIWEM, 2004). Diffuse pollution originates from hundreds or thousands of small sources, or is washed off land with rainfall, making it extremely difficult to quantify inputs to the environment from diffuse sources. However, most diffuse pollutants stem from the use of land for agriculture, industry and from urban land and roadways; thus, examination of these potential sources can provide some insight into the level and types of diffuse pollution a site may be subject to.

In addition to current pollution from both point sources and diffuse sources, it is important to consider the legacy of pollution in an area that may have been left as a result of historical industrial activity. Most metals tend to partition to the sediment and soils, where they do not break down and will remain for many years. Sediment- and soil-dwelling organisms, and those that prey on them, will be exposed to these metals. Sediment-bound metals can also become remobilised into the water column as a result of dredging activities. Maintenance dredging is carried out regularly in the harbours around both the Lymington and Poole sites, potentially remobilising sediment-bound metals, making them more bioavailable to organisms living in the water, and in turn to organisms feeding on them.

Table 4-14 provides a summary of the key non-point sources influencing each of the sites.

Table 4-14 Non-point source influences around the Lymington, Poole and Raby sites

Site	Major non-point source influences
Lymington	Urban and road runoff; Commercial shipping; Recreational boating.
Poole	Urban and road runoff; Agricultural runoff; Recreational boating; Historical industrial pollution.
Raby	Granite geology; Military firing range; Historical mining activity.

In terms of diffuse pollution from roads and urban areas, both the Poole and Lymington sites are likely to be fairly similar. Although the M27 motorway is located in the region of the Lymington site (Section 4.5.2) and there are no motorways in the region of the Poole site, the Poole area has a network of large A-roads, many of which are part dual-carriageway, which are likely to carry a great deal of traffic as they provide links to the coast, and between West Hampshire and East Dorset (Section 4.6.2). The large urban area of the city of Southampton and its suburbs are located within the area round the Lymington site, along with several towns such as Christchurch, Ringwood, Lymington, Newport and Cowes. Around the Poole site, the large towns of Poole and Bournemouth are the main urban areas (for population figures see Sections 4.5.2 and 4.6.2). The Raby site, being very remote, is not subject to a significant level of runoff from urban areas or roads (Section 4.7.2).

The Poole site is more likely to be impacted by agricultural runoff than the Lymington site, as there is more agricultural land around the Poole site and the riverine inputs to the area are also from largely rural catchments, impacted by runoff from agricultural land (Section 4.6.2). For Lymington, the rivers entering the estuary all originate in the New Forest, in which the majority of the land is natural and plantation woodland and heathland, with some agriculture (Section 4.5.2). There is very little agricultural land around the Raby site, thus inputs from agricultural runoff to the area are minimal (Section 4.7.2).

Both Lymington and Poole are important areas with regard to boating and shipping activities, both commercial and recreational (Sections 4.5.4 and 4.6.4). The level of commercial shipping is very high around the Lymington site, particularly in Southampton Water. Poole Harbour has larger, more extensive marina facilities for recreational boating than Lymington; however, there are a large number of smaller marinas within the area of the Lymington site, which may contribute just as much collective pollution to the estuary waters as the fewer, more extensive facilities at Poole. Historically, parts of Poole Harbour were subject to heavy metal pollution as a result of heavy industry in the area, and these industrial activities have left a legacy of heavy metal pollution in the sediments of Poole Harbour (see Section 4.6.3).

The Raby site is likely to be most influenced by natural sources of heavy metals owing to the numerous mineral veins associated with the granite underlying the North Pennines (see Section 4.1), and metal concentrations in the area may be elevated as a result of the historical mining activity. The nearby military firing range may also result in increased concentrations of metals in the local environment and biota (see Section 4.7.3). Runoff from urban areas, roads and

agricultural land is much less significant at the remote Raby site than in the Lymington and Poole areas.

The information provided in this chapter is reviewed alongside the data for concentrations of heavy metals and selenium in black-headed gull eggs in Chapter 5 (Section 5.3.2), in order to assess the potential sources of metal pollution in each area with the concentrations measured in black-headed gull eggs from the Lymington, Poole and Raby sites.

CHAPTER 5. HEAVY METALS AND SELENIUM IN BLACK-HEADED GULL EGGS

5.1 Introduction

Heavy metals are chemical elements that have a specific gravity (a measure of density) at least five times that of water, i.e. 5.0 kg/l or higher (USEPA, 1993). The heavy metals most often implicated in human poisoning are lead, mercury, arsenic, and cadmium. Some heavy metals, such as zinc, copper, iron, and manganese, are required by the body in small amounts (and therefore termed essential metals), but these same elements can be toxic in larger quantities. In the environment, exposure to heavy metals and selenium may occur as a result of a number of anthropogenic activities (see Chapter 4), and these contaminants may be accumulated in the body and become concentrated through food chains, having a direct effect on priority species or habitats. Heavy metals may affect bird populations in a number of ways. Effects may be direct, resulting in increased susceptibility to disease or stress, behavioural effects and decreased reproductive success, or even death (Heinz, 1974; Scheuhammer, 1987; Burger & Gochfeld, 1995b; Heinz *et al.*, 1999). Heavy metal exposure has been shown to affect the reproductive success of birds in a number of field and laboratory tests (Grandjean, 1976; Miles *et al.*, 1993; Eeva & Lehikoinen, 1995), and eggs and young birds are often the most vulnerable to the effects of heavy metals (Burger, 1994). As well as exhibiting direct effects on bird populations, heavy metal pollution may also affect bird populations through effects on the abundance of prey organisms (Bryan & Langston, 1992).

The choice of metals included in this review is not intended to be comprehensive and is based on those elements perceived to be the most environmentally and toxicologically significant (see Chapter 1) and those for which little or no data is available regarding concentrations in bird eggs and feathers, and the effects on reproductive success. This study therefore focuses on the non-essential heavy metals arsenic, cadmium and lead, and the essential metals cobalt, copper, iron, manganese, nickel (essential for some species), vanadium, zinc and the essential semi-metal selenium.

As the toxicological effects of mercury are so well-known, there is an abundance of field data for a wide variety of bird species and the concentrations of mercury in various bird tissues, including eggs and feathers, and the effects of mercury on the breeding success of birds have

been researched extensively. Mercury is also difficult to determine accurately by the analytical technique used in this study - inductively coupled plasma-mass spectrometry (ICP-MS) - as mercury deposits in the sample introduction system and is then released during subsequent analyses (i.e. carry-over). Tin is an important heavy metal in terms of marine pollution, particularly because of its former use in anti-foulant paints applied to the hulls of boats. Although tin was originally included in the suite of metals analysed for in this study, there were issues with the analysis and it was not possible to obtain accurate results for tin concentrations with analysis by ICP-MS. Unfortunately, separate analysis of tin using a different analytical technique was not possible with the limited resources of this project.

The environmental fate and toxicity to birds of the metals covered in this study is dealt with in Section 5.1.1, and the transfer to eggs, fate and behaviour in eggs and toxicity to embryo and chick health is dealt with in Section 5.1.2.

5.1.1 Environmental fate and toxicity

Arsenic

Arsenic is a relatively common element that occurs in air, water, soil and all living tissues (Eisler, 1988b); it is found in the Earth's crust and in nature most often occurs as a compound with sulphide in a variety of complex minerals (Woolson, 1975). Arsenic enters natural waters through weathering and erosion of rocks and soil, through waste streams from industrial processes such as production of alloys and semiconductors, glass and textiles, and from surface runoff from agricultural land or areas where wood preservatives have been used (see also Chapter 4). Entry may also occur as a result of mining, metal smelting and burning of fossil fuels, where arsenic compounds in the atmosphere may be removed from air by wet and dry deposition (HSDB, 2009c), and hence enter natural waters. In water, arsenic occurs in both inorganic and organic forms, with the form depending on a number of variables including pH, organic content, suspended solids and dissolved oxygen concentration (USEPA, 1985). Organic arsenic compounds are mainly found in marine organisms and are much less common in terrestrial species; the majority of organic arsenic in marine animals is present as water-soluble arsenobetaine, and other organisms may be exposed to organic arsenic compounds through a diet containing marine animals (EHC, 2001). Studies have shown that marine organisms such as fish rapidly convert inorganic arsenic administered orally to organic forms in the body

(Penrose, 1975; Oladimeji *et al.*, 1979), with organic arsenicals formed including arsenobetaine and related compounds, di- and trimethylated forms (Oladimeji *et al.*, 1979; Maeda *et al.*, 1990).

The most common forms of arsenic in natural waters are the inorganic dissolved ionic species arsenite (trivalent, As^{3+}) and arsenate (pentavalent, As^{5+}), and the organic methylated forms monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Braman & Foreback, 1973). Soluble inorganic arsenate predominates under normal conditions (USEPA, 1980b; Seyler & Martin, 1989; Pettine *et al.*, 1992); in well-oxygenated water and sediments, nearly all arsenic is present as arsenate, As^{5+} (EHC, 2001). Soluble forms of arsenic move with water and may be carried long distances; however, some forms of arsenic may adsorb onto sediments, depending on the water conditions. In neutral or acidic waters, arsenate partitions to sediments extensively, while arsenite is relatively weakly adsorbed; in waters with high pH, both forms of arsenic are relatively weakly adsorbed (HSDB, 2009c). However, this is not always the case, as partitioning of arsenic is a complex process and depends not only on the form of arsenic and the pH conditions, but also on the type of sediment and interactions with the other materials present (EHC, 2001). Adsorbed arsenic may become remobilised as a result of disruption and mixing through tidal activity, burrowing of benthic organisms or other disruption of sediments (EHC, 2001). Remobilisation from sediments may also occur if conditions become sufficiently reduced for arsenate to form arsenite, or through microbial reduction and methylation.

Although arsenic may be bioaccumulated by organisms, bioaccumulation primarily occurs in algae and, in particular, lower invertebrates, not in higher organisms (EHC, 2001). Arsenic does not tend to increase with trophic level (LeBlanc & Jackson, 1973; Wagemann *et al.*, 1978; Callahan, 1979; Klumpp & Peterson, 1979; Bernhard & Andreae, 1984; Eisler, 1994; Farag *et al.*, 1998; Mason *et al.*, 2000; USEPA, 2003; Williams *et al.*, 2006). For example, an extensive study of the factors affecting bioaccumulation of arsenic found no evidence of bioamplification, and in fact found an overall trend of decreasing arsenic concentrations in organisms with increasing tropic level (Mason *et al.*, 2000). Thus, in general, bioamplification of arsenic in aquatic food chains does not appear to be important in ecological terms.

Arsenic toxicity varies significantly with numerous factors, including form of arsenic, dose, route of administration and species; in general, inorganic arsenic compounds are more toxic than organic compounds, and arsenates are less toxic than arsenites (Gosselin *et al.*, 1984; Eisler, 1988b). Episodes of arsenic poisoning are either acute or subacute, with chronic cases rarely encountered in any species except humans, and early developmental stages of most organisms are more sensitive to arsenic than later stages (Eisler, 1988b).

Arsenic residues in birds tend to be low, with little accumulation reported in tissues of birds even at sites with high environmental concentrations (Martin & Nickerson, 1973; Blus *et al.*, 1977; White *et al.*, 1980; Ohlendorf *et al.*, 1991; Pain *et al.*, 1992; Vermeer & Thompson, 1992; Custer & Hohman, 1994; Guitart *et al.*, 1994; Hothem & Welsh, 1994). For example, Erry *et al.* (1999) examined tissues of raptors from an area in the south west of England with elevated arsenic levels and, although tissue concentrations of arsenic were around three times higher than those of birds from an uncontaminated area, the average concentration of arsenic in the tissues of kestrels (*Falco tinnunculus*) was still relatively low at 0.278, 0.346 and 0.187 mg/kg dry weight for kidney, liver and muscle, respectively (compared to 0.094, 0.121 and 0.057 mg/kg at the uncontaminated site). Arsenic levels were not elevated in the tissues of another two raptor species (sparrowhawk *A. nisus* and barn owl *Tyto alba*) from the same area; the authors suggest that the difference could be attributed to differences in diet and arsenic metabolism between the species. It has been suggested by other authors that the low concentrations measured in birds may be as a result of the ability to rapidly excrete arsenic (Woolson, 1975; Morrissey *et al.*, 2007). In laboratory animals, excrement has been shown to be the primary route of elimination of arsenic (Yamauchi *et al.*, 1990; Hughes *et al.*, 1994; Hughes & Kenyon, 1998), and studies have shown that arsenic is rapidly excreted by chickens, with only 2% of dietary sodium arsenite remaining 60 hours after administration (NAS, 1977a), and 50% of arsenate excreted within 60-63 hours after administration (NRCC, 1978).

Hudson *et al.* (1984) report LD_{50} concentrations for a number of bird species dosed with sodium arsenite through the diet. Data are summarised below in Table 5-1.

Table 5-1 LD_{50} concentration for arsenic as arsenite in bird species (data summarised from Hudson *et al.*, 1984)

Species	Age	LD50 concentration mg As/kg body weight
Mallard *Anas platyryhnchos*	3-4 months	323 (range 149-699)
Pheasant *Phasianus colchicus*	3-4 months	386 (range 221-671)
California quail *Callipepla californica*	9-12 months	47.6 (range 34.3-66.0)

In the mallard, Hudson *et al.* (1984) report an LD_{50} of 323 mg/kg body weight for sodium arsenite (As^{3+}). In the same species, another study also administered arsenic through the diet, as both lead arsenate and sodium arsenite (NAS, 1977a). After 11 days a concentration of 5000

mg As^{5+}/kg in the diet resulted in no mortalities; whereas 50% mortality was observed after only six days at a concentration of 1000 mg As^{3+}. In another study with mallards, ducklings were fed a diet containing 200 mg As^{5+}/kg body weight (as sodium arsenate) for four weeks, and no significant effect on either growth or survival was observed (Hoffman *et al.*, 1992). In a study by Stanley *et al*. (1994), a diet containing 400 mg/kg body weight sodium arsenate significantly reduced mallard duckling growth, but did not affect survival. The results from these studies are in agreement with the general opinion that arsenates are less toxic than arsenites.

Cadmium

Cadmium is found on land, in air and in waters, and transfer between these compartments may be considerable after initial deposition. Entry of cadmium to surface waters occurs as a result of atmospheric fallout, for example from anthropogenic activities such as smelting, refining, burning coals and oils and disposal of sewage sludge (Nriagu & Pacyna, 1988; Furness, 1996), and from surface water runoff and wastewater (HSDB, 2009a; see also Chapter 4). In natural waters, cadmium is found as Cd^{2+} or its hydrates, and as organic and inorganic complexes (Callahan, 1979). As a result, a large fraction of cadmium in the aquatic environment is associated with particulate matter and sediment. However, cadmium can be remobilised and reintroduced into the water column by resuspension of the sediment due to tidal activity or disruption of the estuary bed (for example by storms, flooding or dredging) and with bioturbation by benthic organisms (Zoumis *et al.*, 2001).

Exposure of animals, including birds, to elevated concentrations of the non-essential element cadmium may induce intracellular production of metallothionein, a low molecular-weight protein to which cadmium, and other heavy metals, can be bound and thus rendered less toxic (Furness, 1996). A high accumulation of cadmium can lead to bioamplification, because metallothionein-bound cadmium has a long biological half-life in animals and concentrations tend to increase with age. However, that is not to say that bioamplification always occurs, particularly as cadmium is not lipid soluble, meaning that concentrations will not necessarily increase up the food chain (Furness *et al.*, 1993). Many studies report cadmium concentrations as higher in adults than in juveniles, often ten times and up to as much as 100 times higher in adults than in chicks (Hulse *et al.*, 1980; Stoneburner *et al.*, 1980; Mayack *et al.*, 1981; Stock *et al.*, 1989; Lock *et al.*, 1992).

Although cadmium is accumulated by most organisms, molluscs in particular have been shown to accumulate particularly large amounts (Furness & Rainbow, 1990; Vermeer & Castilla,

1991), and cadmium pollution could be expected to have the greatest effect on those birds feeding on molluscs in enclosed coastal areas with high inputs of cadmium in sewage sludge or from smelter or refinery discharges (Vermeer & Castilla, 1991). The acute toxicity of cadmium to a number of bird species has been examined by Hill *et al*. (1975) and Hill & Camardese (1986). In these studies, the LD_{50} values for cadmium chloride were 2440, 767 and >5000 mg Cd/kg feed for Japanese quail (*Coturnix coturnix japonica*), pheasant (*Phasianus colchicus*) and mallard duck (*Anas platyrhynchos*), respectively. For cadmium succinate, LD_{50} concentrations of 2052, 1411 and >5000 mg Cd/kg feed were reported for Japanese quail, pheasant and mallard duck, respectively. All birds used in these studies were between 10 and 14 days old and were fed the cadmium-dosed diet for five days. In a study by Pritzl *et al*. (1974), domestic chicken (*Gallus* spp.) chicks were fed diets containing 400, 600, 800 or 1000 mg Cd/kg feed. Food consumption and weight gain were decreased at all dose levels, and at concentrations in excess of 400 mg/kg chicks began to lose weight. At the 800 and 1000 mg/kg dose level 100% chick mortality occurred within 20 days; the LD_{50} was calculated to be 565 mg Cd/kg feed.

Cobalt

Traces of cobalt are found in all rocks, minerals and soils and cobalt may be released from these sources by natural weathering (HSDB, 2009d). Anthropogenic sources of cobalt derive mainly from its use as a catalyst in the petrochemical and plastics industries, and in electroplating and alloys (HSDB, 2009d; see also Chapter 4). A large amount of cobalt entering the environment from both natural and anthropogenic sources is released to the atmosphere in association with particulate matter; this cobalt is removed from the atmosphere by wet and dry deposition (WHO, 1998; WHO, 2006), and as a result enter terrestrial and aquatic systems. In nature, cobalt is found as Co^{2+} and Co^{3+}, with Co^{2+} likely to predominate under most normal environmental conditions as it is more stable (Richardson, 1993; HSDB, 2009d). In freshwater the predominant species are Co^{2+} as carbonate, hydroxide and sulphate, and as adsorbed forms, oxide coatings and crystalline sediments; in seawater cobalt is present as cobalt chloride $CoCl^{+}$ and Co^{2+} as carbonate and sulphate (Smith & Carson, 1981). Ultimately, cobalt will partition to sediment, either adsorbing to particles and settling into the sediment, or adsorbing directly to sediment (WHO, 2006). The exact fate of cobalt in natural waters and sediments is complicated by many factors. Partitioning depends on pH, organic matter content and presence of hydrous metal oxides and complexing ligands; a higher pH and greater organic content increase the partitioning of cobalt to soils and sediments (Smith & Carson, 1981). Complexation of cobalt to dissolved organic substances can reduce sediment sorption (Albrecht, 2003), and polluted

waters with higher concentrations of organic pollutants may result in higher concentrations of soluble organic cobalt complexes (Nriagu & Coker, 1980; Smith & Carson, 1981; Szefer *et al.*, 1996; Bargagli, 2000).

As a component of vitamin B_{12}, cobalt is essential for all higher plants and animals (HSDB, 2009d) and adverse effects of cobalt on birds would appear unlikely at concentrations likely to be encountered in the environment (WHO, 2006), which are generally <10 µg/l in surface water and groundwater in populated areas, and <1 µg/l in pristine areas (Smith & Carson, 1981; Hamilton, 1994). Some toxicity studies have been carried out with domestic bird species, for example Diaz *et al*. (1994) administered dietary cobalt at concentrations of 125, 250 and 500 mg/kg feed to one-day old chicks (*Gallus* spp.) for 14 days and found all concentrations to reduce feed intake and weight gain, and a dose-dependant increase in mortality was observed. At a concentration of 100 mg/kg cobalt chloride in the diet of two-week old chicks, a significant adverse effect on growth has been reported (Hill, 1974a); no effect was observed at 50 mg/kg cobalt chloride, but a significant mortality rate was observed in chicks fed 200 mg/kg cobalt chloride for five weeks. In ducklings (*Anas* spp.), a dietary concentration of 200 mg/kg cobalt chloride resulted in lesions but no mortality, and a significant mortality occurred at a dietary concentration of 500 mg/kg cobalt chloride fed for 28 days (Van Vleet *et al.*, 1981).

Copper

Copper is widely distributed in the environment and is essential for the normal growth and metabolism of all living organisms (Schroeder *et al.*, 1966; Carbonell & Tarazona, 1994). Although abnormally low concentrations of copper may induce a nutritional deficiency, at abnormally high concentrations copper is among the most toxic of the heavy metals to freshwater and marine biota (Schroeder *et al.*, 1966). The largest sources of copper are anthropogenic in origin, such as emissions from mining and smelting activities, industrial emissions and effluents, municipal wastes, sewage sludge, coal burning and use in antifoulant paints (Nriagu, 1989; Eisler, 1998b; see also Chapter 4). A large amount of copper entering the environment from both natural and anthropogenic sources is released to the atmosphere in association with particulate matter (WHO, 1998); this copper is removed from the atmosphere by wet and dry deposition (WHO, 1998), and as a result enters terrestrial and aquatic systems. The fate of copper in the aquatic environment is influenced by several processes including formation of complexes, sorption to hydrous metal oxides, clays and organic materials, and bioaccumulation (Stiff, 1971; Callahan, 1979). Much of the copper discharged to water is in

particulate form and tends to settle out or be adsorbed by organic matter, hydrous iron, manganese oxides and clay in the sediment or water column (WHO, 1998).

In the natural environment, copper occurs in three oxidation states: as elemental copper, Cu^0, and as Cu^+ and Cu^{2+} in sulphides, arsenites, chlorides and carbonates (ATSDR, 1990). In aquatic systems, copper is expected to exist in the dissociated form as ions or as insoluble salts (Sharma & Millero, 1988). The speciation and bioavailability of copper in aquatic systems depends on a number of factors, including water hardness and alkalinity, ionic strength, pH, redox potential, complexing ligands and suspended particulate matter (Callahan, 1979; WHO, 1998). However, in general, copper may be found as elemental copper or the cuprous ion, Cu^+ in anaerobic waters, and in aerobic waters most copper is present as the cupric ion, Cu^{2+} (Sharma & Millero, 1988); thus, the cupric ion is the one generally encountered in natural waters (Eisler, 1998b). The free cupric ion is the most readily available and toxic inorganic species of copper; however, in natural waters only a very small percentage of the copper present will exist as the free aquo ion, $Cu(H_2O)_6^{2+}$, and most copper is adsorbed on suspended particles or complexed with various ligands (Florence & Batley, 1980; Bryan & Langston, 1992). The major chemical species of copper in seawater are copper hydroxychloride, copper hydroxide and copper carbonate (USEPA, 1980c).

In comparison to lower organisms, birds and mammals are relatively resistant to copper (Eisler, 1998b) and, in general, birds retain a very small portion of copper ingested (Bryan & Langston, 1992). Experiments with domestic poultry show that 350 mg/kg Cu in the diet leads to a reduction in weight gain of chicken (*Gallus* spp.) chicks (NAS, 1977b), and in two separate studies growth impairment was observed at a 500 mg/kg dose and damage to the gizzard lining was evident in chicken (Poupoulis & Jensen, 1976) and turkey (*Meleagris gallopava*; Kashani *et al.*, 1986) poults. In the latter study, a dietary dose of 120 mg/kg Cu inhibited growth of turkey poults for the first eight weeks, but not during the following 16 weeks. In another study with turkey poults fed diets containing 100-800 mg/kg Cu, no adverse effects on survival were observed at any concentration and growth reduction occurred only at the highest dose of 800 mg/kg diet (Supplee, 1964).

Iron

Iron is the second most abundant metal on earth and is found in all soil, rocks and minerals (Cotton *et al.*, 1999). Elemental iron is rarely found in nature due to its high reactivity (Huebers, 1991), but iron compounds are released through natural weathering of soil and rocks

(HSDB, 2008b). Iron compounds may also be released as a result of the mining and processing of iron ores, via emissions from iron and steel industries, industrial and domestic sewage effluents, and vehicle exhausts (Kirk-Othmer, 1995; see also Chapter 4). Iron compounds emitted as particles in the atmosphere may reach terrestrial and aquatic systems via wet or dry deposition, and runoff from highways will also result in inputs of iron compounds to aquatic systems. As previously mentioned, elemental iron (Fe^0) is rarely found in nature due to its high reactivity (Huebers, 1991); the most common oxidation states of iron are Fe^{2+} and Fe^{3+}, with Fe^{3+} expected to be the form of iron present under most aerobic environmental conditions (Kirk-Othmer, 1995). In water, Fe^{3+} is expected to hydrolyse or form complexes with organic matter and ligands (Cotton *et al.*, 1999); adsorption of iron depends on the amount of organic matter present and pH, with an increase in either factor leading to increased adsorption of iron (Gerritse, 1981).

Iron is an essential element required by all forms of life and is present in all foods of plant or animal origin (WHO, 2004b). Iron toxicosis is not a common problem in most animals, most likely because of its limited absorption and uptake when intakes are high (NAS, 1980). However, if intakes are sufficiently high, particularly if over a sustained period, signs of iron toxicity can occur as tissues become overloaded and free (reactive) iron levels become high enough to cause peroxidative damage (i.e. iron toxicosis results in the formation of reactive oxygen which causes cell damage; Underwood & Suttle, 1999). Although extremely large doses of iron can be fatal, in most animals iron toxicosis requires very high concentrations of iron along with intakes of cobalt, zinc, manganese or copper (Abdel-Mageed & Oehme, 1990). Studies with birds are few and appear to be limited to domestic species. No mortality nor sub-lethal effects such as lesions were observed in 2-day old domestic chicken chicks (*Gallus* spp.) fed ferrous sulphate orally up to 100 mg (Wallner-Pendleton *et al.*, 1986). However, in a study with 3-day old chicks of the same species, 180, 240 and 300 mg ferrous sulphate (36, 48 or 60 mg iron) administered orally resulted in 6.6, 16.1 and 26.5% mortality, respectively, within 24 hours (Pescatore & Harter-Dennis, 1989). An LD_{50} of 357 mg ferrous sulphate for chicks of domestic chicken species has been reported (NAS, 1980).

Lead

Lead is a naturally occurring element, found in combination with other elements as lead compounds in rocks and soils; minor natural sources of lead emissions include silicate dusts, volcanic emissions, forest fires and the decay of radon (EA, 2007). As a result of its extensive use in iron, steel and non-ferrous metal production, coal-fired power stations, the chemical

industry and as an additive in fuels (EA, 2007), as well as lead mining, ore-processing, smelting, manufacture of lead compounds and use of lead metals, alloys and compounds, lead is ubiquitous in air, water and soil, and in both rural and urban environments (Lide, 2008).

Lead reaches the aquatic environment through industrial and municipal discharge, in atmospheric deposition, in road runoff and from weathering processes in areas of natural lead mineralisation (USEPA, 1980a; Harrison & Laxen, 1981; see also Chapter 4). Sorption is an important process in removing lead from the water column: Pb^{2+} is the stable ionic species of lead and in the environment forms complexes of low solubility with major anions such as hydroxide, carbonate, sulphide and sulphate ions, which limit solubility (HSDB, 2009a). As a result, a large fraction of lead in the aquatic environment is associated with particulate matter and sediment. However, lead can be remobilised and reintroduced into the water column by tidal activity or disruption of the estuary bed (for example, by dredging) and by bioturbation by benthic organisms (Morel *et al.*, 1975; Schulz-Baldes *et al.*, 1983).

Lead is a highly toxic, non-essential heavy metal that acts as a non-specific poison affecting all body systems (Pain, 1996). It enters the food chain through air, water and soil, and can bioaccumulate to some extent in organisms that are high in the food chain (Burger & Gochfeld, 2000c), with older organisms usually containing the greatest body burdens (Eisler, 1988a). In general, lead does not bioamplify up the food chain (Eisler, 1988a). Exposure to lead can cause neurobehavioural, hematologic, nephrotoxic and reproductive effects in humans and other animals (Cory-Slechta *et al.*, 1983; Needleman *et al.*, 1990; Rice, 1996).

In a review of lead concentrations measured in birds, Eisler (1988a) reports that concentrations were highest in birds from urban locations and those near mining and smelting facilities. Lead residues were also greatest in older birds, in sexually mature females and in waterfowl that had ingested lead shot pellets. In birds, lead accumulates primarily in the bones and, of the soft tissues, the kidneys accumulate the highest concentrations (Custer *et al.*, 1984). The results of studies on the chronic toxicity of dietary lead in birds indicate that young altricial birds (i.e. chicks that hatch with little or no down, eyes closed, incapable of departing the nest and fed by the parents; Nice, 1962) have a greater susceptibility to lead than adults (Scheuhammer, 1987). For example, 448 mg/kg metallic lead administered to adult kestrels (*F. sparverius*) through the diet did not affect survival or body weight, however, nestling kestrels fed comparable concentrations of lead exhibited decreased growth rates and increased mortality compared to controls (Hoffman *et al.*, 1985). Growth impairment of nestlings was associated with kidney

lead concentrations of greater than 6 mg/kg wet weight, and survival impairment was associated with kidney lead concentrations of greater than 15 mg/kg.

Toxic and sublethal effects of lead and its compounds on birds vary widely with species, age, sex, form of lead and the dose administered (Eisler, 1988a). Metallic lead is not toxic to birds except at very high doses when administered in the form of powder, although it is highly toxic to birds when given as lead shot (EHC, 1989); however, the use of lead shot is now restricted in many countries, including the UK. Inorganic lead is only toxic to birds at a high dietary dosage. In a study investigating the effects of metallic and inorganic lead, Hill and Camardese (1986) fed Japanese quail (*C. coturnix japonica*) diets containing different forms of lead and saw no effect on survival, food consumption or other signs of toxicity in birds fed 5000 mg/kg lead as lead nitrate, lead subacetate or metallic lead. However, in the same study an LD_{50} of 2761 mg/kg lead as lead arsenate was reported, indicating that lead in the form of lead arsenate is more toxic than metallic lead, lead nitrate or lead subacetate.

Information on the effects of organolead compounds is limited and, as previously mentioned, effects vary greatly with age, sex, dose and form. Organic forms of lead are more toxic than inorganic forms as they are more bioavailable and lipid-soluble, and as a result are more readily absorbed by the body (WHO, 1989; ATSDR, 2007). Tetraethyl- and tetramethyllead are well-known organolead compounds, owing to their extensive use as fuel additives, both historically in leaded petrol for motor vehicles (EHC, 1989), and currently in aviation and premium grade fuels (Proctor *et al.*, 2004). Tetraethyl- and tetramethyllead are readily converted to triethyl- and trimethyllead in water and in animals (Howell *et al.*, 1986; EHC, 1989; Cukrowska *et al.*, 2007). Trialkyllead salts are ten to 100 times more toxic to birds than inorganic lead salts (Forsyth *et al.*, 1985). The LD_{50} for a single oral dose of tetraethyllead administered to mallards (*A. platyrynchos*) is reported as 107 mg/kg body weight (Bellrose, 1951); in Japanese quail (*C. coturnix japonica*) the LD_{50} for a single dose of tetraethyllead has been reported as around a quarter of the concentration in mallards - 24.6 mg/kg body weight (Hudson *et al.*, 1984). A dose of 28 mg triethyllead per kg body weight fed to starlings (*Sturnus vulgaris*) over a period of 11 days resulted in 100% mortality by the sixth day, and the same was observed for birds dosed with an equal concentration of trimethyllead (Osborn *et al.*, 1983). A recurring incident of massive bird kills in estuaries near to industrial plants manufacturing leaded compounds was reported in the late 1970s and early 1980s; affected birds contained elevated lead levels, mostly in the form of alkyllead (EHC, 1989).

Manganese

Manganese occurs naturally in the environment in the form of numerous minerals, and enters the environment through natural weathering of rocks and soil, via windblown dust and through volcanic emissions (HSDB, 2009e). The major anthropogenic sources of environmental manganese result from the use of manganese compounds in the manufacture of alloys, steel and iron products, burning of fossil fuels and incineration of sewage sludge (WHO, 2004a; see also Chapter 4). If released to air, manganese compounds will exist in the particulate phase and may be removed from the air by wet and dry deposition (HSDB, 2009e), and as a result enter terrestrial and aquatic systems. Manganese may also enter natural waters via mine wastes, sewage, industrial discharges and run-off from agricultural land, landfill and highways (Lagerwerff, 1967).

Manganese is multi-valent and can exist in 11 oxidation states ranging from -3 to +7, the most common being Mn^{2+}, Mn^{4+} and Mn^{7+} (WHO, 2004a). Under environmental conditions, Mn^{2+} is the most stable oxidation state and, in this oxidation state, manganese does not form strong complexes with organic matter and ligands and is therefore mainly present as soluble, bioavailable Mn^{2+} in the water column (Bodek *et al.*, 1988). However, manganese is readily transferable between solution and the solid phase (both sediment and suspended particulates) in response to changes in redox conditions (Spencer & Brewer, 1971; Emerson *et al.*, 1979). Insoluble Mn^{3+} and Mn^{4+} compounds may be formed, but in natural waters in the presence of organic matter, Mn^{3+} and Mn^{4+} compounds will usually reduce to soluble manganous (Mn^{2+}) compounds (Bodek *et al.*, 1988). In freshwater, soluble Mn^{2+} is the major species, and in seawater manganese is present mostly in the particulate form as Mn^{4+} precipitated to manganese dioxide, with manganous salts such as manganese chloride, manganese sulphate, manganese carbonate and manganese hydrogen carbonate also present in appreciable quantities (WHO, 1981; Bodek *et al.*, 1988). Manganese dioxide is poorly soluble in water and exists in the suspended particulate form, while most of the manganous salts present are soluble, with the exception of manganese carbonate which has a relatively low solubility in water (WHO, 1981; WHO, 2004a). In estuarine waters where resuspension of sediment occurs as a result of tides, storm events, commercial and recreational boating and sometimes dredging activities, levels of particulate manganese are higher than in oceanic waters (WHO, 1981) as manganese in the sediments is oxidised to form Mn^{4+}.

Manganese is an essential trace element and is necessary for the formation of connective tissue and bone, for growth, carbohydrate and lipid metabolism and reproductive functions

(Underwood, 1971; NAS, 1973). The importance of manganese for birds has been recognised since 1936, when it was demonstrated experimentally that manganese could prevent the bone-deforming disease perosis in chickens (*Gallus* spp.; Wilgus *et al.*, 1936). Subsequent research with birds has shown that manganese is essential for the activation of numerous enzymes and vital for growth, egg production and embryo development (Underwood, 1971).

The toxicity of manganese varies according to the chemical form to which the organism is exposed, with Mn^{2+} being between two and a half and three times more toxic than Mn^{3+} (WHO, 1981). Manganese toxicity studies with birds are extremely limited. Burger and Gochfeld (1995b) observed significant adverse effects on growth and behaviour of herring gull (*L. argentatus*) chicks following a single intraperitoneal injection of manganese acetate at 25 mg Mn/kg body weight. Sierra *et al*. (1998) exposed feral pigeons (*Columba livia*) to manganese tetroxide dust at 239 μg Mn/m^3 for seven hours a day, five days a week for 13 weeks and report no significant toxic effects at this exposure level.

Nickel

In addition to natural weathering of rock and soils, nickel from various industrial processes may enter natural waters. Industrial waste streams, residues and effluents from waste water treatment plants, wet and dry deposition of atmospheric nickel and runoff from landfill areas and highways will all contribute to nickel entering the aquatic environment (WHO, 1991; see also Chapter 4). Nickel can exist in a number of oxidation states ranging from -1 to +4, but normally occurs in the elemental state Ni^0 and as Ni^{2+} (Eisler, 1998a). In natural waters the Ni^{2+} species is the most common, and is most commonly present as the aquo ion $(Ni(H_2O)_6)^{2+}$ (WHO, 1991). Nickel is one of the most mobile heavy metals in the aquatic environment and occurs as soluble salts adsorbed onto or associated with clay particles, organic matter and other substances (Callahan, 1979; Eisler, 1998a). The fate of nickel in water is affected by pH, type and concentration of ligands and the presence and availability of organic matter (Eisler, 1998a); in general, in pristine environments more nickel is adsorbed to sediment, but in polluted waters with more prevalent organic matter more nickel will be in the soluble phase (Callahan, 1979).

Nickel is essential for the normal growth of many species of microorganisms and plants (WHO, 1991) and several vertebrate species including chickens, cows, goats, pigs, rats and sheep (NAS, 1975; WHO, 1991). Adverse effects of excess nickel have been reported in a number of organisms, including birds. Newly hatched domestic chickens (*Gallus* spp.) fed more than 300 mg/kg nickel in the diet exhibited reduced growth rates, and with dietary doses in excess of 500

mg/kg Ni mortality occurred (Outridge & Scheuhammer, 1993). Young birds appear to be more sensitive than adults: dietary doses of nickel in excess of 800 mg/kg resulted in significant mortality in newly hatched mallard (*A. platyrhynchos*) ducklings, but no mortality, nor evidence of systemic or reproductive toxicity, was observed in adults fed at the same dose level (Eastin & O'Shea, 1981). In another study with mallard ducklings, nickel was administered through the diet as nickel sulphate for 90 days (Cain & Pafford, 1981). No effects on growth or survival were observed in the 200 or 800 mg/kg dose groups, although a lower bone density was measured in females at day 60 in the 800 mg/kg group. The birds fed 1200 mg/kg exhibited significant weight loss and reduced bone densities, and 71% mortality occurred by day 60.

Selenium

Although it is not a heavy metal, selenium is commonly considered alongside heavy metals because it has many metal-like characteristics and is often released from the same sources as heavy metals (Eisler, 1985b; WHO, 1986). Selenium has been shown to have a detrimental effect on the breeding success of birds (see below and Section 5.1.2), further warranting its inclusion in this study.

The major source of environmental selenium is weathering of natural rock. The largest anthropogenic source of selenium is coal combustion, with other sources including waste and emissions from mining and milling operations, base metal smelting and refining, selenium refining, burning of coal, oil and solid waste (HSDB, 2008a; see also Chapter 4). In nature, selenium is found in the selenide (Se^{2-}), elemental selenium (Se^{0}), selenite (Se^{4+}) and selenate (Se^{6+}) oxidation states (HSDB, 2008a). Selenium found in natural waters is mostly the result of weathering of seleniferous rock (Callahan *et al.*, 1979). Selenide exists as hydrogen selenide and in a number of metallic selenides. Hydrogen selenide is a gas at room temperature and decomposes rapidly in air to form elemental selenium and water, while selenides of heavy metals, such as iron selenide, occur naturally in many minerals and tend to be insoluble (Johnson, 1976; NAS, 1976). Elemental selenium is virtually insoluble in water (Heinz, 1996), and thus is not present in water in any appreciable amount. Selenite is soluble in water, but will bind tightly to iron and aluminium oxides, and thus will be partitioned to sediments and, like elemental selenium, is not present in the water column in any appreciable amount (WHO, 1986). However, tightly bound selenium in sediments may be remobilised into the water column if the sediment conditions become sufficiently oxidised for selenite to oxidise to selenate (Alemi *et al.*, 1988), for example through bioturbation by benthic organisms, or disruption/mixing of the sediment through dredging processes, storms and strong tides. Selenate is soluble and stable in

water and is the most common form of selenium found in natural waters, particularly in alkaline waters, and is thus the most bioavailable and potentially environmentally dangerous form of the element (WHO, 1986). Biomethylation of both inorganic and organic selenium compounds by microorganisms may also occur, producing methylated compounds such as gaseous dimethyl selenide and dimethyl diselenide (Callahan *et al.*, 1979).

Selenium is essential in small amounts for birds and other wildlife to maintain good health; it is part of the body's antioxidant defence system, playing an important role in boosting the immune system, and is important in thyroid hormone metabolism and in reproduction. However, the range of dietary selenium that provides adequate but non-toxic amounts is narrow compared to the ranges for some other essential elements (Heinz, 1996). At high concentrations selenium has been shown to cause behavioural abnormalities, reduced reproductive success and even mortality (Eisler, 1985b; Ohlendorf *et al.*, 1986; Ohlendorf *et al.*, 1988; Ohlendorf, 1989; Heinz, 1996).

The toxicity of selenium depends largely on its chemical form; organoselenium compounds are believed to be major forms in plants and animals (Heinz, 1996). In general, most plants convert most of the inorganic selenium to which they are exposed into organic selenium, in particular the protein amino acid, selenomethionine, in non-Se accumulating plants, and non-proteinaceous amino acids such as selenocysteine in Se-accumulating plants (Spallholz & Hoffman, 2002). Animals such as insects and fish that feed on these plants will deposit selenomethionine in their proteins in place of methionine, and may also make selenocysteine (Ohlendorf, 1989). Aquatic birds that feed on a mixed diet of plant foods, insects, small crustaceans and fish, such as black-headed gulls, are therefore expected to ingest selenium mainly in its organic form, as selenomethionine, as well as lesser amounts of selenium as selenocysteine (Spallholz & Hoffman, 2002). Of the inorganic ions, selenite (Se^{4+}) and selenate (Se^{6+}) are toxic to birds, but organic selenides pose the greatest hazard (Heinz, 1996). Most laboratory toxicity data for selenium looks at the highly toxic organic form of selenium, selenomethionine, which, as previously mentioned, is believed to be the major form of selenium in the diet of aquatic birds (Heinz, 1996; Spallholz & Hoffman, 2002).

In birds, the egg and embryo stages of development are more sensitive to selenium toxicity than the health and survival of young and adult birds (Heinz *et al.*, 1987; Heinz *et al.*, 1988; Hoffman & Heinz, 1988); for example, selenomethionine becomes particularly embryotoxic and teratogenic at dietary levels of over 4 mg/kg in mallards, but higher concentrations are needed before toxicity to other mallard life stages will occur (Hoffman, 2002). Adult mallards (*A.*

platyrhynchos) fed a diet containing selenium as selenomethionine for 14 weeks readily accumulated selenium in the liver in a dose-dependant manner, and dietary selenium at the highest concentration of 32 mg/kg diet (wet weight) resulted in 10% mortality (Hoffman *et al.*, 1991). Adult American coots (*Fulica americana*) from Kesterson Reservoir in California were found to be emaciated and had severe lesions, leading to an investigation into the possible causes (Ohlendorf *et al.*, 1988). The reservoir was used as a hydrologic sink for drainage from a large agricultural area, with several ponds serving as evaporation and holding basins for this agricultural drainage water (Freedman, 1995). Inflow waters to the reservoir had average concentrations of 0.3 mg/l (compared to 0.05 mg/l selenium in water furthest downstream of the inflow), and mean selenium concentrations in the livers of adult coots collected at the site were 28 and 29 mg/kg wet weight, respectively, which were about ten times greater than those of adults sampled from a nearby control area. This comparison would suggest that the effects observed in the Kesterton Reservoir birds can be attributed to selenium toxicosis.

Owing to the sensitivity of embryonic stages of birds to selenium toxicity, and the resulting impacts of selenium on reproductive success, the majority of studies concentrate on the effects of selenium toxicity on eggs and embryos. This area of selenium toxicity will be dealt with in Section 5.1.2, 'Selenium'.

Vanadium

Vanadium compounds are widely distributed in the earth's crust and are present in numerous minerals and in association with fossil fuels, although elemental vanadium does not occur in nature (ATSDR, 2004). Vanadium compounds are released to the environment as a result of natural weathering of rocks and soils, volcanic emissions and wind-blown dust (ATSDR, 2004), and are also released to the atmosphere as a result of the combustion of fossil fuels and solid waste, then entering the environment via wet and dry deposition (WHO, 2001a). In addition, vanadium may enter surface waters via industrial and wastewater effluents and runoff from landfill, agricultural land and highways (see also Chapter 4). Although vanadium can exist in a number of oxidation states ranging from -1 to +5, in the environment vanadium exists as V^{3+}, V^{4+} and V^{5+} (WHO, 2001a). In most natural waters V^{3+} and V^{4+} are rapidly oxidised to pentavalent forms of vanadium (V^{5+}) and, under oxidising conditions, most vanadium will be present as V^{5+}; under reducing conditions, most vanadium will be present as V^{4+} (HSDB, 2008c). Both V^{4+} and V^{5+} compounds are expected to adsorb strongly to organic matter and form complexes, and only about 0.001% of vanadium entering marine waters is estimated to persist in soluble form (HSDB, 2008c), with the majority in suspension or adsorbed (WHO,

2001a). The most commonly used form of vanadium is vanadium pentoxide (V_2O_5), in which vanadium is in the +5 oxidation state (WHO, 2001a); following entry into water, vanadium pentoxide is likely to convert to vanadium oxides such as metavanadate, tetravanadate and pyrovanadate sodium salts (Crans *et al.*, 1998).

Studies with laboratory rodents suggest that pentavalent forms of vanadium are the most potent and the most readily bioaccumulated in the body (Parker & Sharma, 1978; Llobet & Domingo, 1984). Although vanadium is an essential element for chicks with effects of vanadium deficiency including reduced growth, impairment of reproduction and disturbance of the lipid metabolism (WHO, 2001a), exposure to excess concentrations of vanadium can lead to toxic effects. Data on vanadium toxicity on birds are limited and largely confined to domestic bird species. In newly hatched chickens (*Gallus* spp.), dietary exposure to 100 mg/kg body weight vanadium as ammonia metavanadate for 4 weeks impaired growth, and 200 mg/kg resulted in 20% mortality (Hafez & Kratzer, 1976). In mallards (*A. platyrhynchos*) fed 100 mg/kg body weight vanadium as vanadyl sulphate over a 12 week period concentrations of vanadium accumulated in tissues was low and no signs of toxicity were observed (White & Dieter, 1978). This is most likely due to the lower potency and uptake of trivalent forms of vanadium such as vanadyl sulphate compared to pentavalent forms such as metavanadate. Rattner *et al*. (2006) examined the toxicity of vanadium compounds to mallard ducks and Canada geese (*Branta canadensis*). Following a seven day single oral dose trial with mallards, an LD_{50} of 113 mg/kg body weight is reported for vanadium pentoxide. Sodium metavanadate was more potent, with an LD_{50} of 75.5 mg/kg body weight reported for mallards, and Canada geese were more sensitive than mallards with an LD_{50} of 37.2 mg/kg body weight for sodium metavanadate. The same authors also conducted chronic exposure experiments with the same species and found that exposure of mallards to increasing dietary concentrations of sodium metavanadate (38.5 to 2651 mg/kg) over 67 days resulted in mild intestinal haemorrhage, food avoidance and weight loss.

Zinc

Zinc is found in almost all minerals in the earth's crust and is an essential trace element for life that is found in all living organisms (Ohnesorge & Wilhelm, 1991). Natural weathering of rocks and soils leads to the natural release of zinc to the environment (NAS, 1979). Zinc also enters the environment from anthropogenic sources as a result of its many uses (see Chapter 4). In the environment, zinc is present exclusively as the Zn^{2+} ion, and may be found in several chemical forms in the aquatic environment, the most common of which is a mixture of the toxic aquo ion - $(Zn(H_2O)_6)^{2+}$ - and various metal-inorganic and metal-organic complexes (Eisler, 1993). The

majority of zinc in the marine and estuarine environment will eventually become partitioned into the sediments and suspended solids as metal complexes (Eisler, 1993; HSDB, 2009b). However, zinc can be remobilised into the water column by bioturbation and resuspension of the sediment due to tidal activity or disruption of the estuary bed, for example by storm events, flooding or dredging (Zoumis *et al.*, 2001; UK Marine SAC, 2009). Soluble chemical species of zinc are the most bioavailable and the most toxic and, as previously mentioned, the aquo ion predominates over most other dissolved species in the aquatic environment and is thought to be the most toxic (Eisler, 1993).

Zinc poisoning has been documented in a number of different animal species, but is of particular importance in the aquatic environment because the gills of fish are physically damaged by high concentrations of zinc (NAS, 1979), and aquatic populations are frequently devastated as a result of zinc pollution. Significant adverse effects on growth, reproduction and survival have been documented for sensitive species of aquatic plants, invertebrates and vertebrates at water concentrations of between 10 and 25 µg/l zinc (Eisler, 1993). Exposure of animals to acutely high, or chronically low, concentrations of zinc may induce intracellular production of metallothionein, binding zinc and rendering it less toxic (Richards, 1989; Eriksen *et al.*, 1990).

Ducks (*Anas* spp.) have shown reduced survival when fed diets containing in excess of 3000 mg Zn/kg feed (Gasaway & Buss, 1972; NAS, 1979). Domestic chickens (*Gallus* spp.) appear to be more resistant than ducks, with no mortality observed in laying hens fed diets containing 10000, 20000 and 30000 mg Zn/kg feed (Decuypere *et al.*, 1988; Verheyen *et al.*, 1990). Adults also appear to be more resistant than chicks: domestic chicken chicks showed slight reduction in growth after being fed 2000 mg Zn/kg feed, and significant growth reduction, but no mortality, at 3000 mg Zn/kg feed (NAS, 1979). A dose of 4000 mg Zn/kg diet fed to day-old chicks had no effects on growth or survival (Oh *et al.*, 1979); at a dose of 8000 mg/kg feed zinc 80% mortality occurred, with the survivors' growth significantly reduced, and 100% mortality occurred at 16000 mg/kg dose.

5.1.2 Heavy metals and selenium in eggs

Concentrations in seabird eggs have been reported for many different metals, by several authors (Anderlini *et al.*, 1972; Parslow *et al.*, 1972; Blus *et al.*, 1977; Parslow & Jeffries, 1977; Hulse *et al.*, 1980; King *et al.*, 1983; Honda *et al.*, 1986; Renzoni *et al.*, 1986). However, fewer studies are available which examine the concentrations of metals other than lead, cadmium, mercury and selenium in eggs and relate these to tissue concentrations or environmental

exposure. Dauwe *et al*. (2005) report higher concentrations of a number of essential elements including cobalt, copper, nickel and zinc in eggs, compared to concentrations in the ovary and other tissues of female birds. Of the other metals studied, arsenic and mercury concentrations were relatively high in eggs compared with those in internal tissues, while silver, cadmium and lead concentrations were relatively low compared with those in internal tissues. In a study examining concentrations of 17 trace elements in the eggs of waterbirds, eggs were found to be good bioindicators of arsenic, lead, zinc, copper, vanadium and zinc (Lam *et al.*, 2005) due to consistent correlations between the concentrations of these elements in eggs and in marine sediments.

Although eggs often represent heavy metal exposure of the adults that have laid them, metals are not sequestered equally in eggs and, for many metals, concentrations in the egg contents do not adequately reflect body burdens or dietary intakes (Becker, 1989). For example cadmium appears not to be transferred by the female to the eggs in easily measurable quantities (Burger, 1993; Burger, 1994). However, this is not to say that transfer does not occur, just that it is often found to be very low (Sell, 1975; Furness *et al.*, 1993). Eggs have been used in monitoring pollution by a number of authors as a means of providing a reflection of metal uptake from local exposure, and transfer from the laying female to eggs has been demonstrated in a number of studies (Burger & Gochfeld, 1991; Burger & Gochfeld, 1993; Burger & Gochfeld, 1995a; Burger & Gochfeld, 1996), with eggs shown to represent local exposure of the adults that have laid them for a number of metals. Mercury concentrations in eggs have been widely reported and eggs have been shown to reflect the uptake of mercury from local foraging more closely than internal tissues from adult birds (Parslow & Jeffries, 1977; Barrett *et al.*, 1985). It has proved difficult to assess toxic effects of metals on bird populations and some birds may have high metal burdens for reasons of natural accumulation or detoxification processes unrelated to pollution (Furness *et al.*, 1993); thus high metal concentrations in birds do not necessarily indicate pollution (Murton *et al.*, 1978; Muirhead & Furness, 1988). The concentrations of some metals in eggs have been examined in a very limited number of studies, if at all, and further research is required in this area given the evidence available that such pollutants may have harmful effects.

The following section examines the transfer of arsenic, cadmium, cobalt, copper, iron, lead, manganese, nickel, vanadium, zinc and selenium to eggs and the effects of these metals on reproductive success, including egg laying, embryo and chick health, development and survival.

Arsenic

Table 5-2 provides a summary of literature data for arsenic concentrations in seabird eggs. Data are for dry weight concentrations from studies worldwide, undertaken from 1990 onwards; 'egg contents' refers to combined yolk and albumen.

Table 5-2 Summary of literature data for arsenic concentrations in seabird eggs

Species	Location	Mean concentration ppm (dry weight)	Study year	Reference
Herring gull ***Larus argenatatus***	New Jersey, USA	0.126	2000	Burger, 2002
Great black-backed gull ***Larus marinus***	New Jersey, USA	0.1	2000	Burger, 2002
Common tern ***Sterna hirundo***	New Jersey, USA	0.195	2000	Burger, 2002
Forster's tern ***Sterna forsterii***	New Jersey, USA	0.19	2000	Burger, 2002
Bridled tern ***Sterna anaethetus***	Hong Kong	1.384	2000-2002	Lam et al., 2005
Black skimmer ***Rynchops niger***	New Jersey, USA	0.529	2000	Burger, 2002

Transfer of arsenic from the laying bird to eggs has been demonstrated in a number of studies. Holcman and Stibilj (1997) fed hens diets containing 7.5, 15 or 30 mg As^{3+}/kg (as arsenic oxide) for a period of 19 days. Eggs collected and analysed contained mean concentrations of 0.2, 0.42 and 0.96 mg As/kg dry weight in egg yolk, and 0.06, 0.14 and 0.3 mg As/kg dry weight albumen, for the three exposure concentrations, respectively. Stanley *et al*. (1994) also report arsenic to be accumulated in a dose-dependant manner in a number of samples, including eggs, taken from mallard ducks fed a diet containing 25, 100 and 400 mg As^{5+}/kg, as sodium arsenate, with concentrations in whole eggs ranging from 0.46 to 3.6 mg/kg.

In a field study comparing concentrations of heavy metals in eggs of passerine species between polluted and unpolluted sites, Dauwe *et al*. (1999) found significantly higher arsenic concentrations in eggs from the polluted site, with nine eggs collected at the polluted site and five eggs at the reference site. This report suggests that laying birds exposed to high levels of arsenic excrete excess arsenic into eggs. In another study with passerines, Dauwe *et al*. (2005) found arsenic concentrations in the eggshell to be high compared with internal tissue concentrations for the ten birds sampled, although concentrations in the egg contents and eggshell were poorly correlated with concentrations in both internal tissues and feathers. Lam *et al*. (2005) found significant correlations between arsenic concentrations measured in sediment and in the eggs of waterbird species in the same area (nine eggs from each of three species sampled; $p < 0.001$), suggesting eggs to be good indicators for monitoring arsenic.

Data regarding the effects of arsenic on reproductive success in birds is limited. Domestic chickens (*Gallus* spp.) fed a diet containing up to 30 mg As^{3+}/kg body weight (as arsenic oxide) for 19 days showed no significant change in feed consumption, body weight, egg production and average egg weight (Holcman & Stibilj, 1997). Following a single oral dose of 100 mg As^{3+}/kg body weight (as sodium arsenite) to female mallards (*A. platyrhynchos*), eggshell thickness was reduced within three days but recovered to normal after five days (Haegele & Tucker, 1974). However, the authors suggest that this temporary reduction in eggshell thickness may be accounted for by decreased food consumption during this period, although food consumption was not measured. In chicken embryos, a mortality rate for arsenite of 34% is reported at a dose range of 0.01-1.0 mg As^{3+}/embryo, whereas the mortality rate for arsenate administered at the same concentration is much lower, at only 8% (NRCC, 1978). As previously mentioned (Section 5.1.2), the evidence from this study would suggest that arsenic as arsenite is more toxic to birds, including embryonic stages, than arsenate. Birge and Roberts (1976) report survival rates of embryos of domestic chicken eggs treated by yolk injection with a number of metals at various concentrations. Arsenic as arsenite resulted in 35% mortality at the lowest dose of 0.001 mg/kg egg; at 0.01 mg/kg 46% mortality was observed, and of the surviving embryos a further 2% exhibited gross malformations. At doses of 1.0 mg/kg and above, no embryos survived.

Cadmium

In birds, cadmium accumulates in the kidneys and concentrations in other tissues tend to be very much lower (Thompson, 1990); thus, the only routine means of accurately measuring cadmium in bird populations is considered to be the sampling of tissues from the adults (Furness *et al.*, 1993). Eggs have been used as a means of monitoring cadmium pollution (Furness *et al.*, 1993; Burger & Gochfeld, 1995a), providing a reflection of uptake from local foraging; however, concentrations in eggs are usually very low (Burger & Gochfeld, 1991), and embryotoxic effects unlikely (Furness, 1996). Despite high concentrations of cadmium in the kidneys of many seabirds, concentrations reported in eggs are usually less than 0.7 µg/g wet weight (Osborn *et al.*, 1979; Honda *et al.*, 1986; Renzoni *et al.*, 1986). Table 5-3 provides a summary of literature data for cadmium concentrations in seabird eggs. Data are for dry weight concentrations from studies worldwide, undertaken from 1990 onwards; 'egg contents' refers to combined yolk and albumen.

Table 5-3 Summary of literature data for cadmium concentrations in seabird eggs

Species	Location	Mean concentration ppm (dry weight)	Study year	Notes	Reference
Herring gull ***Larus argenatatus***	Long Island, USA	0.01	1992	egg contents	Burger, 1994
	Long Island, USA	0.05	1992	eggshell	Burger, 1994
	New Jersey, USA	0.005	2000		Burger, 2002
Great black-backed gull ***Larus marinus***	New Jersey, USA	0.005	2000		Burger, 2002
Black-tailed gull ***Larus crassirostris***	Japan	0.021	1999-2001	egg contents	Agusa *et al*., 2005
	Japan	0.013	1999-2001	eggshell	Agusa et al., 2005
Common tern ***Sterna hirundo***	New Jersey, USA	0.004	2000		Burger, 2002
Roseate tern ***Sterna dougallii***	Long Island, USA	0.2	1992	egg contents	Burger, 1994
	Long Island, USA	0.1	1992	eggshell	Burger, 1994
Forster's tern ***Sterna forsterii***	New Jersey, USA	0.002	2000		Burger, 2002
Bridled tern **Sterna anaethetus**	Hong Kong	0.002	2000-2002		Lam *et al*., 2005
Black skimmer ***Rynchops niger***	New Jersey, USA	0.002	2000		Burger, 2002
Short-tailed albatross ***Phoebastria albatrus***	Japan	0.007	2002	egg contents	Ikemoto *et al*., 2005
	Japan	0.013	2002	eggshell	Ikemoto *et al*., 2005
Black-footed albatross ***Phoebastria nigripes***	Japan	0.007	2002	egg contents	Ikemoto *et al*., 2005
	Japan	0.097	2002	eggshell	Ikemoto *et al*., 2005

Anomalously high concentrations of cadmium in eggs, for example a concentration of 75.0 µg/g found in sooty tern (*S. fuscata*) eggs from Hawaii (Stoneburner & Harrison, 1981), are thought to be a possible result of the breakdown of the apparent cadmium transfer barrier to the eggs if other tissues become overloaded (Hutton, 1981). Laboratory experiments have shown transfer of cadmium to eggs to be very low, regardless of the amount consumed (Sell, 1975; Furness *et al.*, 1993), and thus low concentrations of these metals in eggs of wild birds do not necessarily reflect a low dietary intake. On the other hand, a number of studies have demonstrated transfer of cadmium from laying female to eggs (Burger & Gochfeld, 1991; Burger & Gochfeld, 1993; Burger & Gochfeld, 1995a; Burger & Gochfeld, 1996), and evidence from both field and laboratory studies is conflicting.

Some evidence exists suggesting that cadmium concentrations in eggshells may be high, and concentrations of cadmium have been reported to be higher in the eggshell than egg contents (Dauwe *et al.*, 2005). As cadmium is not lipid soluble, eggshells might provide a means of monitoring cadmium concentrations which cannot be adequately measured using egg contents (Furness *et al.*, 1993); many studies look only at concentrations in egg contents, and this may go some way towards explaining the fact that most studies find concentration in eggs (referring to egg contents) to be very low. In a field study comparing concentrations of heavy metals in eggs of passerine species between polluted and unpolluted sites, Dauwe *et al.* (1999) found

significantly higher cadmium concentrations in eggs from the polluted site - in both eggshells and egg contents - suggesting that laying birds exposed to high levels of cadmium excrete excess cadmium into eggs. In a later study with passerines, Dauwe *et al*. (2005) found cadmium concentrations in the eggshell to be correlated with concentrations in tail feathers, back feathers and liver. Cadmium concentrations in egg contents were not correlated with any of the internal tissues or other feathers examined. Data for partitioning of cadmium, and other metals, between eggshell and contents is provided in Table 5-12.

There is no evidence from field studies of pelagic seabird species, many of which have been reported to contain high kidney cadmium concentrations (100 mg/kg or more) that eggshell thinning occurs as a result of exposure to high levels of cadmium. In laboratory tests, eggshell thinning has been demonstrated in chickens as a result of dietary exposure to cadmium (Furness, 1996), although the transfer of dietary cadmium to eggs is considered to be very low (Sell, 1975; Burger & Gochfeld, 1991; Burger, 1993; Gochfeld, 1997; Mora, 2003). Leach *et al*. (1979) found that, at the 48 mg/kg dose given to hens through the diet, approximately 90 mg/kg cadmium was accumulated in the kidney but an increase of cadmium in the egg was also observed, egg production was halved and egg shell thickness decreased in comparison to hens fed the control diet. However, when the authors replicated the study no differences in eggshell quality between the control and cadmium-dosed birds were found. Dietary cadmium has been demonstrated to suppress egg production in mallards at a concentration of 48 mg/kg (Leach *et al.*, 1979); however, in another study cadmium fed to adult mallards at concentrations up to 200 mg/kg for 90 days had no effect on egg production (White & Finley, 1978). Sell (1975) fed chickens a diet containing 60 mg/kg cadmium as $CdCl_2$ and found that the hens fed this diet ate less and laid fewer eggs than hens given the control diet. Conversely, White and Finley (1978) found dietary cadmium fed to laying hens at a concentration of 200 mg/kg to suppress egg production, but found no effect on egg laying in hens fed dosed at cadmium concentrations lower than 200 mg/kg. In terms of embryotoxicity, Birge and Roberts (1976) report survival rates of embryos of domestic chicken eggs treated by yolk injection with a number of metals at various concentrations. At the lowest dose of 0.001 mg cadmium/kg egg, 34% mortality occurred; at 0.05 mg/kg 52% mortality occurred, and at doses of 5.0 mg/kg and above, no embryos survived.

Cobalt

Table 5-4 provides a summary of literature data for cobalt concentrations in seabird eggs. Data are for dry weight concentrations from studies worldwide, undertaken from 1990 onwards; 'egg contents' refers to combined yolk and albumen.

Table 5-4 Summary of literature data for cobalt concentrations in seabird eggs

Species	Location	Mean concentration ppm (dry weight)	Study year	Notes	Reference
Black-tailed gull ***Larus crassirostris***	Japan	0.043	1999-2001	egg contents	Agusa *et al*., 2005
	Japan	0.74	1999-2001	eggshell	Agusa *et al*., 2005
Bridled tern ***Sterna anaethetus***	Hong Kong	0.026	2000-2002	egg contents	Lam *et al*., 2005
	Hong Kong	0.398	2000-2002	eggshell	Lam *et al*., 2005
Short-tailed albatross ***Phoebastria albatrus***	Japan	0.007	2002	egg contents	Ikemoto *et al*., 2005
	Japan	0.26	2002	eggshell	Ikemoto *et al*., 2005
Black-footed albatross ***Phoebastria nigripes***	Japan	0.007	2002	egg contents	Ikemoto *et al*., 2005
	Japan	0.205	2002	eggshell	Ikemoto *et al*., 2005

There are very few studies examining the transfer of cobalt from the laying bird to the egg. Agusa *et al*. (2005) calculated maternal transfer rates for a number of trace elements, defined as the percentage of a trace element burden in eggs to the whole body burden (internal tissues, feathers and eggs). The transfer rate of cobalt to eggs was calculated as 30%. In a field study with passerines, Dauwe *et al*. (2005) found cobalt concentrations in both the eggshell and contents to be high compared with internal tissue concentrations for the 10 birds sampled ($p = 0.004$), suggesting cobalt is sequestered into eggs. However, concentrations in the egg contents and eggshell were poorly correlated with concentrations in both internal tissues and feathers.

No data could be found regarding the reproductive effects, embryotoxicity or teratogenicity of cobalt or cobalt compounds.

Copper

Table 5-5 provides a summary of literature data for copper concentrations in seabird eggs. Data are for dry weight concentrations from studies worldwide, undertaken from 1990 onwards; 'egg contents' refers to combined yolk and albumen.

Table 5-5 Summary of literature data for copper concentrations in seabird eggs

Species	Location	Mean concentration ppm (dry weight)	Study year	Notes	Reference
Black-tailed gull ***Larus crassirostris***	Japan	4.14	1999-2001	egg contents	Agusa *et al*., 2005
	Japan	0.535	1999-2001	eggshell	Agusa *et al*., 2005
Audouin's gull ***Larus audouinii***	Spain	2.58	1992	egg contents	Morera *et al*., 1997
	Spain	2.14	1992	eggshell	Morera *et al*., 1997
Bridled tern ***Sterna anaethetus***	Hong Kong	3.92	2000-2002	egg contents	Lam *et al*., 2005
Short-tailed albatross ***Phoebastria albatrus***	Japan	4.84	2002	egg contents	Ikemoto *et al*., 2005
	Japan	0.766	2002	eggshell	Ikemoto *et al*., 2005
Black-footed albatross ***Phoebastria nigripes***	Japan	4.66	2002	egg contents	Ikemoto *et al*., 2005
	Japan	0.784	2002	eggshell	Ikemoto *et al*., 2005

Literature regarding the sequestering of copper into eggs is conflicting. In a field study of American peregrine falcon (*F. peregrinus anatum*) eggs, copper concentrations were significantly greater in eggs from unsuccessful nests compared to successful nests (sample sizes of 31, 26 and 32 over three separate years; Ambrose *et al.*, 2000). However, concentrations of iron and mercury were also significantly higher in eggs from unsuccessful nests and thus it cannot be concluded that elevated copper concentrations in the eggs were solely responsible for the lack of success of the nests. Indeed, in another study Dauwe *et al*. (1999) report no difference in copper concentrations between eggs of passerine species taken from a polluted site compared to those from a non-polluted site (sample size: nine eggs from polluted site, five eggs from reference site), while concentrations of other metals were significantly higher in eggs from the polluted site. The authors suggest that the concentrations of essential elements such as copper do not differ between polluted and unpolluted sites because, being essential elements, their levels are controlled homeostatically (i.e. regulated internally to maintain a stable, constant condition). However, in a later study with passerines, Dauwe *et al*. (2005) found copper concentrations in egg contents to be high compared with internal tissue concentrations for the ten birds sampled, although concentrations in the egg were poorly correlated with concentrations in both internal tissues and feathers. Lam *et al*. (2005) found significant correlations between copper concentrations measured in sediment and in the eggs of waterbird species in the same area (nine eggs from each of three species sampled), suggesting eggs to be good indicators for monitoring copper. In a laboratory study with domestic chickens (*Gallus* spp.), laying hens were fed diets supplemented with various concentrations of copper, up to 800 mg/kg body weight (Chiou *et al.*, 1997). Copper concentration in the egg contents increased with increasing dietary dose, up to 400 mg/kg copper, and after a normal diet was resumed

copper concentrations in eggs declined significantly. This would suggest that copper is indeed excreted into eggs by the laying hen.

Studies investigating the effects of copper and copper compounds on the reproductive success of birds are scarce. The embryotoxic and teratogenic potential of copper gluconate and cupric chloride has been investigated in the developing chick embryo by Verrett (1973; 1974; 1976). The author found that copper gluconate was embryotoxic even at the lowest levels tested of 1 mg/kg (1973) and a teratogenic effect also occurred in the developing chick embryo (1974). In a later study (1976), cupric chloride at concentrations as low as 0.25 mg/kg was shown to have an embryotoxic effect, but not a teratogenic effect. Jackson (1977) observed no effects on feed intake, water consumption and egg production in hens fed 480 mg/kg copper in the diet; however, a 50% reduction in egg production was observed at 960 mg/kg. In a later study, Jackson *et al*. (1979) report a decrease in weight gain and egg production at a dose of 600 mg/kg, a 50% reduction in egg production at 800 mg/kg, and a complete halt to egg production after 14 days at the highest dose rate of 1920 mg/kg copper. No literature data could be found regarding the impacts of copper on eggshell thickness.

Iron

No studies were found reporting concentrations of iron in seabird eggs from field studies, and no data was found regarding maternal transfer of iron to eggs from the laying bird. In a field study of American peregrine falcon (*F. peregrinus anatum*) eggs, iron concentrations were significantly greater in eggs from unsuccessful nests compared to successful nests (mean egg concentrations 115 and 85 mg/kg dry weight, respectively), with samples of 31, 26 and 32 eggs taken over three separate years (Ambrose *et al.*, 2000). However, concentrations of copper and mercury were also significantly greater in eggs from unsuccessful nests and thus it cannot be concluded that elevated iron concentrations in the eggs were solely responsible for the lack of success of the nests. No differences were found between eggs sampled in terms of eggshell thickness.

No literature data could be found reporting the sequestering of iron to eggs, nor the impacts of elevated iron concentrations reproductive success.

Lead

Eggs have been used as a means of monitoring lead pollution (Furness *et al.*, 1993; Burger, 1994; Burger & Gochfeld, 1995a; Burger, 2002; Ikemoto *et al.*, 2005; Lam *et al.*, 2005), providing a reflection of uptake from local foraging. Table 5-6 provides a summary of literature data for lead concentrations in seabird eggs. Data are for dry weight concentrations from studies worldwide, undertaken from 1990 onwards; 'egg contents' refers to combined yolk and albumen.

Table 5-6 Summary of literature data for lead concentrations in seabird eggs

Species	Location	Mean concentration ppm (dry weight)	Study year	Notes	Reference
Herring gull ***Larus argenatatus***	New Jersey, USA	0.273	2000		Burger, 2002
Great black-backed gull ***Larus marinus***	New Jersey, USA	0.227	2000		Burger, 2002
Common tern ***Sterna hirundo***	New Jersey, USA	0.164	2000		Burger, 2002
Roseate tern ***Sterna dougallii***	Long Island, USA	2.3	1992	egg contents	Burger, 1994
	Long Island, USA	1.2	1992	eggshell	Burger, 1994
Forster's tern ***Sterna forsterii***	New Jersey, USA	0.056	2000		Burger, 2002
Bridled tern ***Sterna anaethetus***	Hong Kong	0.01	2000-2002	egg contents	Lam *et al*., 2005
Black skimmer ***Rynchops niger***	New Jersey, USA	0.334	2000		Burger, 2002
Short-tailed albatross ***Phoebastria albatrus***	Japan	0.011	2002	eggshell	Ikemoto *et al*., 2005
Black-footed albatross ***Phoebastria nigripes***	Japan	0.01	2002	egg contents	Ikemoto *et al*., 2005
	Japan	0.039	2002	eggshell	Ikemoto *et al*., 2005

In spite of being used as a means of monitoring lead pollution, the degree to which birds can sequester lead in eggs is unclear (Pattee, 1984; Burger & Gochfeld, 1988; Burger & Gochfeld, 1991; Burger & Gochfeld, 1993). Some authors have failed to detect elevated concentrations of lead in the eggs of experimentally dosed birds (Pattee, 1984) or birds exposed to high concentrations in the environment (Spahn & Sherry, 1999), while others have shown elevated concentrations (Haegele *et al.*, 1974; Maedgen *et al.*, 1982), and transfer of lead in bird eggs has been demonstrated in various studies by Burger and Gochfeld (1991; 1993; 1995a; 1996). For example, these authors found correlations between the concentrations of lead in egg contents and tissues of the females that produced them ($p = 0.02$) in a sample of 24 pairs of common terns (*S. hirundo*) and an egg from each of their nests (Burger & Gochfeld, 1991). Dauwe *et al.* (1999) found significantly higher lead concentrations in eggs of great tits (*Parus major*) from polluted sites (nine eggs sampled) in comparison with unpolluted sites (five eggs sampled) - in both eggshells and egg contents (egg contents: polluted site 2.0 μg/g lead dry weight, unpolluted site 0.13 μg/g ($p = 0.001$); eggshell: polluted site 15 μg/g; unpolluted site 0.37 μg/g ($p = 0.007$)).

These results suggest that laying birds exposed to high levels of lead excrete excess lead into eggs. In a later study with passerines, Dauwe *et al*. (2005) found lead concentrations in the egg contents and shell were correlated with concentrations in tail feathers and concentrations in the egg contents were correlated with concentrations in the liver and the stomach contents, from a sample of 10 female great tits (*P. major*; p-values not reported). Lam *et al*. (2005) found significant correlations between lead concentrations measured in sediment and in the eggs of waterbird species in the same area (three species, nine eggs sampled from each species; $p < 0.001$), suggesting eggs are good indicators for monitoring lead.

The failure of some studies to detect elevated concentrations of lead in the eggs of lead-dosed birds may be due to the lead concentrations often being below detection limits (usually less than 0.4 µg/g; Reid and Hacker, 1982; Renzoni *et al*., 1986), or in some cases studies have examined egg contents only, and as lead concentrations have been reported to be higher in eggshell than egg contents (Mora, 2003; Dauwe *et al.*, 2005), possibly as a result of interaction of lead with calcium metabolism (Scheuhammer, 1987), this may not provide an accurate reflection of lead concentration in the egg. However, other studies have reported lead concentrations to be higher in egg contents (Burger, 1994), so this relationship is unclear. Data for partitioning of lead, and other metals, between eggshell and contents is provided in Table 5-12.

In general, concentrations of inorganic lead salts below 100 mg/kg in the diet cause few significant reproductive effects in birds (Scheuhammer, 1987). For example, a dose of 50 mg/kg metallic lead fed to American kestrels (*Falco sparverius*) for a period of six months produced no adverse effects on egg laying, fertility or eggshell thickness (Pattee, 1984). Haegle *et al*. (1974) dosed female mallards (*A. platyrhynchos*) with 100 mg/kg lead as a mixture of lead carbonate, lead oxide and lead sulphate, via the diet. No significant effect on eggshell thickness was found after 85 days of treatment. Edens *et al*. (1976) investigated the effects of dietary lead acetate on reproductive success in Japanese quail (*C. coturnix japonica*). In the study, chicks were reared from hatching on diets containing lead acetate and growth rate and egg production were examined. No effect was observed on the growth rate of the chicks with the exception of the highest dose rate of 1000 mg/kg lead acetate. Egg production was suppressed even at the lowest dose of 1 mg/kg lead acetate in the diet and suppression of egg production increased with increasing lead dose; at the highest dose level of 1000 mg/kg lead in the diet, egg production was almost completely suppressed and the few eggs that were produced were soft-shelled or shell-less. The hatch rate of eggs laid by birds fed 100 or 1000 mg/kg was significantly reduced.

Tetraethyllead administered via the diet has been shown to have no effect on eggshell thickness in mallard ducks (*A. platyrhynchos*) or Japanese quail (*C. coturnix japonica*) at a dose of 6 mg/kg body weight over six days (Haegele & Tucker, 1974). As previously mentioned, tetraethyllead may also be converted to trialkyllead, the salts of which are ten to 100 times more toxic to birds than inorganic lead salts, and tend to accumulate in lipophilic soft tissues in the yolk and developing embryo (Forsyth *et al.*, 1985). Although results suggest that trialkyllead compounds are very toxic to young and adult birds (Osborn *et al.*, 1983), no studies could be found examining the effects of trialkyllead compounds on eggs and embryo development.

Manganese

Table 5-7 provides a summary of literature data for manganese concentrations in seabird eggs. Data are for dry weight concentrations from studies worldwide, undertaken from 1990 onwards; 'egg contents' refers to combined yolk and albumen.

Table 5-7 Summary of literature data for manganese concentrations in seabird eggs

Species	Location	Mean concentration ppm (dry weight)	Study year	Notes	Reference
Herring gull ***Larus argenatatus***	Long Island, USA	5.5	1992	egg contents	Burger, 1994
	Long Island, USA	8.2	1992	eggshell	Burger, 1994
	New Jersey, USA	1.622	2000		Burger, 2002
Great black-backed gull ***Larus marinus***	New Jersey, USA	1.651	2000		Burger, 2002
Black-tailed gull ***Larus crassirostris***	Japan	1.82	1999-2001	egg contents	Agusa *et al*., 2005
	Japan	1.13	1999-2001	eggshell	Agusa *et al*., 2005
Audouin's gull ***Larus audouinii***	Spain	1.69	1992	egg contents	Morera *et al*., 1997
	Spain	0.29	1992	eggshell	Morera *et al*., 1997
Common tern ***Sterna hirundo***	New Jersey, USA	2.29	2000		Burger, 2002
Roseate tern ***Sterna dougallii***	Long Island, USA	4.2	1992	egg contents	Burger, 1994
	Long Island, USA	4.3	1992	eggshell	Burger, 1994
Forster's tern ***Sterna forsterii***	New Jersey, USA	1.702	2000		Burger, 2002
Bridled tern ***Sterna anaethetus***	Hong Kong	2.636	2000-2002		Lam *et al*., 2005
Black skimmer ***Rynchops niger***	New Jersey, USA	1.282	2000		Burger, 2002
Short-tailed albatross ***Phoebastria albatrus***	Japan	0.405	2002	egg contents	Ikemoto *et al*., 2005
	Japan	0.552	2002	eggshell	Ikemoto *et al*., 2005
Black-footed albatross ***Phoebastria nigripes***	Japan	0.865	2002	egg contents	Ikemoto *et al*., 2005
	Japan	0.533	2002	eggshell	Ikemoto *et al*., 2005

Very few studies have examined the transfer of manganese from the laying bird to the egg; however, the limited evidence suggests that manganese is excreted into eggs (Burger, 1994). In

a field study with passerines, Dauwe *et al*. (2005) found manganese concentrations in egg contents to be relatively high compared with some internal tissue concentrations (concentration in egg contents 2.48 µg/g dry weight, concentration in muscle tissue 0.94 µg/g, heart 1.65 µg/g). However, egg concentrations were low compared to other tissues (bone 5.08 µg/g, kidney 4.85 µg/g, stomach 3.86 µg/g), and egg concentrations were poorly correlated with concentrations in both internal tissues and feathers. Concentrations of manganese in egg contents were consistently higher than those in shells (means 2.48 µg/g and 0.67 µg/g, respectively).

In laboratory studies with mammals, excess manganese exposure causes mortality and decreased fertility (Grey & Laskey, 1980; Laskey *et al.*, 1982), decreases in motor activity (Ingersoll *et al.*, 1995) and nervous system dysfunction (Mergler, 1986). Studies with birds are much fewer, but manganese has been demonstrated to cause neurobehavioural effects (Burger & Gochfeld, 1995b; Burger & Gochfeld, 2002) and sublethal exposure of avian embryos to manganese causes teratogenic effects such as abnormally small, poorly developed or twisted limbs, haemorrhage and neck defects; an LD_{50} of 765 µg/egg has been determined for manganese (Gilani & Alibhai, 1990).

Nickel

Literature data for nickel concentrations in seabird eggs is very limited, and concentrations were found only for eggshell; from one study. Table 5-8 provides a summary of this data.

Table 5-8 Summary of literature data for nickel concentrations in seabird eggs

Species	Location	Mean concentration ppm (dry weight)	Study year	Notes	Reference
Curlew ***Numenius arquata***	Vammala, Finland	1.14	1996	eggshell	Currie and Valkama, 1998
	Kauhava, Finland	1.14	1996	eggshell	Currie and Valkama, 1998

In a study with female laying great tits, Dauwe *et al*. (2005) found nickel concentrations in both egg contents and eggshells to be high compared with internal tissue concentrations in a sample of 10 female great tits ($p = 0.004$), suggesting maternal transfer of nickel to eggs does occur. However, concentrations in the egg and shell were poorly correlated with concentrations in both internal tissues and feathers, so the degree to which nickel is sequestered into eggs by laying birds is still unclear.

Only one study could be found reporting the toxicity of nickel to bird embryos. For chick embryos injected with a single dose of nickel as nickel chloride, 50% mortality occurred within

18 days at a dose of 3.6 mg Ni/kg embryo (Ridgway & Karnofsky, 1952). No studies could be found reporting the effects of nickel on eggshell thickness.

Selenium

The concentration of selenium in wild birds eggs has been examined by a number of authors (Ohlendorf *et al.*, 1986; Ohlendorf, 1989; Williams *et al.*, 1989a; Burger, 1994; Braune *et al.*, 2001; Braune *et al.*, 2002; Burger, 2002; Ikemoto *et al.*, 2005; Lam *et al.*, 2005). A summary of literature data for selenium concentrations in seabird eggs is provided in Table 5-9. Data in this table are for dry weight concentrations from studies worldwide, undertaken from 1990 onwards; 'egg contents' refers to combined yolk and albumen.

Table 5-9 Summary of literature data for selenium concentrations in seabird eggs

Species	Location	Mean concentration ppm (dry weight)	Study year	Notes	Reference
Herring gull ***Larus argenatatus***	Long Island, USA	2.9	1992	egg contents	Burger, 1994
	Long Island, USA	0.4	1992	eggshell	Burger, 1994
	New Jersey, USA	1.836	2000		Burger, 2002
Glaucous gull ***Larus hyperboreus***	Prince Leopold Island, Canadian Arctic	2.7	1993		Braune *et al*., 2002
	Browne Island, Canadian Arctic	2.2	1993		Braune *et al*., 2002
	Richardson River, Canadian Arctic	1.1	1993		Braune *et al*., 2002
	Anderson River, Canadian Arctic	2.6	1993		Braune *et al*., 2002
Great black-backed gull ***Larus marinus***	New Jersey, USA	1.543	2000		Burger, 2002
Black-tailed gull ***Larus crassirostris***	Japan	3.3	1999-2001	egg contents	Agusa *et al*., 2005
	Japan	0.42	1999-2001	eggshell	Agusa *et al*., 2005
Black-legged kittiwake ***Rissa tridactyla***	Canadian Arctic	4.38	1993		Braune *et al*., 2001
	Canadian Arctic	2.43	1998		Braune *et al*., 2001
	Prince Leopold Island, Canadian Arctic	4.4	1993		Braune *et al*., 2002
	Coburg Island, Canadian Arctic	4.4	1993		Braune *et al*., 2002
Common tern ***Sterna hirundo***	New Jersey, USA	2.046	2000		Burger, 2002
Roseate tern ***Sterna dougallii***	Long Island, USA	4.2	1992	egg contents	Burger, 1994
	Long Island, USA	0.005	1992	eggshell	Burger, 1994
Forster's tern ***Sterna forsterii***	New Jersey, USA	1.688	2000		Burger, 2002
Bridled tern ***Sterna anaethetus***	Hong Kong	4.386	2000-2002		Lam *et al*., 2005
Black skimmer ***Rynchops niger***	New Jersey, USA	1.834	2000		Burger, 2002
Northern fulmar ***Fulmarus glacialis***	Canadian Arctic	4.01	1993		Braune *et al*., 2001
	Canadian Arctic	3.34	1998		Braune *et al*., 2001

Table 5-9 cont.

Species	Location	Mean concentration ppm (dry weight)	Study year	Notes	Reference
Thick-billed murre ***Uria lomvia***	Canadian Arctic	2.57	1993		Braune *et al*., 2001
	Canadian Arctic	2.2	1998		Braune *et al*., 2001
	Prince Leopold Island, Canadian Arctic	2.6	1993		Braune *et al*., 2002
	Coburg Island, Canadian Arctic	2.1	1993		Braune *et al*., 2002
	Digges Island, Canadian Arctic	2.3	1993		Braune *et al*., 2002
	Coats Island, Canadian Arctic	2.4	1993		Braune *et al*., 2002
	Prince Leopold Island, Canadian Arctic	2.2	1998		Braune *et al*., 2002
	Coats Island, Canadian Arctic	2.3	1998		Braune *et al*., 2002
Black guillemot ***Cepphus grylle***	Prince Leopold Island, Canadian Arctic	2.2	1993		Braune *et al*., 2002
	Nuvuk Island, Canadian Arctic	2.2	1993		Braune *et al*., 2002
	Walrus Island, Canadian Arctic	2.7	1993		Braune *et al*., 2002
Short-tailed albatross ***Phoebastria albatrus***	Japan	2.7	2002	egg contents	Ikemoto *et al*., 2005
	Japan	0.08	2002	eggshell	Ikemoto *et al*., 2005
Black-footed albatross ***Phoebastria nigripes***	Japan	4.5	2002	egg contents	Ikemoto *et al*., 2005
	Japan	0.15	2002	eggshell	Ikemoto *et al*., 2005

The transfer of selenium to eggs is considerable (Focardi *et al.*, 1988), and the main form of dietary selenium to which aquatic birds are exposed - selenomethionine - readily accumulates in the protein of egg albumen (Spallholz & Hoffman, 2002).

Studies suggest that reproductive success is more sensitive to selenium toxicity than the health and survival of young and adult birds. Numerous studies have investigated the effects of embryonic exposure to selenium; embryo deformities and hatching failure occur when selenium concentrations in egg contents exceed *ca*. 3 ppm on a wet-weight basis and eggs provide a sensitive measure for evaluating hazards to birds (Heinz, 1996). Birge and Roberts (1976) report survival rates of embryos of domestic chicken (*Gallus* spp.) eggs treated by yolk injection with a number of metals at various concentrations. At the lowest dose of 0.001 mg/kg egg, selenium as selenate caused 36% mortality; at 0.01 mg/kg 57% mortality was observed, and at doses of 1.0 mg/kg and above no embryos survived. Exposure to excess dietary selenium concentrations has been associated with decreased egg weight, decreased egg production and hatchability and a high incidence of deformed embryos with missing or distorted eyes, beaks, wings and feet (Harr, 1978; Ort & Latshaw, 1978). As previously mentioned, although selenite (Se^{4+}) and selenate (Se^{6+}) are toxic to birds, organic selenides pose the greatest hazard (Heinz, 1996), and selenomethionine has been demonstrated to be more effective than sodium selenite at raising the selenium content of tissues and eggs (Moksnes, 1983). Reduced hatching of eggs

was recorded in Japanese quail (*C. coturnix japonica*) fed between 6 and 12 mg/kg dietary selenite (El-Begearmi *et al.*, 1977), and in mallards (*A. platyrhynchos*) fed 10 mg/kg selenium as selenomethionine or 25 mg/kg as sodium selenite a 40-44% decrease in the total number of eggs hatching has been reported (Heinz *et al.*, 1987; Hoffman & Heinz, 1988).

In wild birds, severe reproductive effects have been reported in ducks (*Anas* spp.), American coot (*F. americana*) and other species of aquatic birds nesting at irrigation drain water ponds in the Kesterton National Wildlife Refuge in the San Joaquin Valley, California (Ohlendorf *et al.*, 1986; Ohlendorf, 1989; Williams *et al.*, 1989a). The concentration of selenium in the water of these ponds was abnormally high - around 300 μg/l - while concentrations of other metals (silver, chromium, arsenic, cadmium, mercury, lead and zinc) were low (Ohlendorf *et al.*, 1986). A number of field studies were carried out and these indicated a high frequency (up to 65%) of deformities in embryos and hatchlings of the nesting aquatic birds, including missing or abnormal beaks, eyes, wings, legs or feet, and multiple deformities were observed in many cases (Ohlendorf *et al.*, 1986; Ohlendorf, 1989; Williams *et al.*, 1989a). Selenium concentrations were 2-110 mg/kg in egg contents and 19-130 g/kg in livers of birds, which equated to between 7 and 130 times higher than concentrations measured in nearby control areas, and several pathological and biochemical symptoms of selenium toxicosis were observed in the adult wild birds (Ohlendorf *et al.*, 1988). It was concluded that selenium was the probable cause of poor reproduction and developmental abnormalities in the aquatic nesting birds, due to interference with the reproductive process.

Vanadium

Table 5-10 provides a summary of literature data for vanadium concentrations in seabird eggs. Note: data provided are for dry weight concentrations from studies worldwide, undertaken from 1990 onwards; ‘egg contents’ refers to combined yolk and albumen.

Table 5-10 Summary of literature data for vanadium concentrations in seabird eggs

Species	Location	Mean concentration ppm (dry weight)	Study year	Notes	Reference
Black-tailed gull ***Larus crassirostris***	Japan	0.041	1999-2001	egg contents	Agusa *et al*., 2005
	Japan	0.011	1999-2001	eggshell	Agusa *et al*., 2005
Bridled tern ***Sterna anaethetus***	Hong Kong	0.096	2000-2002		Lam *et al*., 2005
Short-tailed albatross ***Phoebastria albatrus***	Japan	0.013	2002	egg contents	Ikemoto *et al*., 2005
	Japan	0.008	2002	eggshell	Ikemoto *et al*., 2005
Black-footed albatross ***Phoebastria nigripes***	Japan	0.023	2002	egg contents	Ikemoto *et al*., 2005
	Japan	0.008	2002	eggshell	Ikemoto *et al*., 2005

Only one study could be found regarding the transfer of vanadium to bird eggs. Lam *et al*. (2005) found a strong pattern linking concentrations of vanadium measured in sediment with concentrations in the eggs of waterbird species in the same area (three species, nine eggs sampled for each species), suggesting eggs to be good indicators for monitoring vanadium.

Vanadium is an essential element for birds and vanadium deficiency causes reduced growth, impairment of reproduction and disturbance of the lipid metabolism (WHO, 2001a). In general, it would seem that vanadium deficiency is more likely to have an adverse effect on reproductive success than vanadium toxicosis. However, some studies with domestic chickens (*Gallus* spp.) report adverse effects of excess vanadium on reproductive success of birds. A reduction in egg production, decreased egg weight and decreased eggshell weight has been observed for hens fed vanadium at a dose of 30-40 mg/kg diet (Ousterhout & Berg, 1981; Davis *et al.*, 1995; Bressman *et al.*, 2002). Conversely, in another study with hens fed vanadium supplemented feed up to 100 mg/kg diet, egg weight and shell thickness were unaffected, even at the highest concentration of 100 mg/kg feed. It should also be noted that the effects on the egg may be, at least in part, due to the significant reduction in feed consumption of the laying hen during the experimental period.

Excess vanadium in the diet of laying hens (*Gallus* spp.) has also been shown to significantly reduce hatchability, at doses of 25 mg/kg diet (Kubena *et al.*, 1980) and 40 mg/kg diet (Bressman *et al.*, 2002). An increase in embryo mortality has also been reported as a result of laying hens fed vanadium-supplemented feed at a dose of 60 mg/kg diet (Bressman *et al.*, 2002).

Zinc

Table 5-11 provides a summary of literature data for zinc concentrations in seabird eggs. Data are for dry weight concentrations from studies worldwide, undertaken from 1990 onwards; 'egg contents' refers to combined yolk and albumen.

Table 5-11 Summary of literature data for zinc concentrations in seabird eggs

Species	Location	Mean concentration ppm (dry weight)	Study year	Notes	Reference
Black-tailed gull ***Larus crassirostris***	Japan	65.3	1999-2001	egg contents	Agusa *et al*., 2005
	Japan	0.778	1999-2001	eggshell	Agusa *et al*., 2005
Audouin's gull ***Larus audouinii***	Spain	58.3	1992	egg contents	Morera *et al*., 1997
	Spain	6.58	1992	eggshell	Morera *et al*., 1997
Bridled tern ***Sterna anaethetus***	Hong Kong	47.6	2000-2002		Lam *et al*., 2005
Short-tailed albatross ***Phoebastria albatrus***	Japan	62.6	2002	egg contents	Ikemoto *et al*., 2005
	Japan	5.61	2002	eggshell	Ikemoto *et al*., 2005
Black-footed albatross ***Phoebastria nigripes***	Japan	71	2002	egg contents	Ikemoto *et al*., 2005
	Japan	3.39	2002	eggshell	Ikemoto *et al*., 2005

A number of studies have demonstrated maternal transfer of zinc to eggs (Williams *et al.*, 1989b; Bryan *et al.*, 2003), and eggs have been used for monitoring zinc pollution to provide a reflection of uptake and exposure. Lam *et al*. (2005) found significant correlations between zinc concentrations measured in sediment and in the eggs of waterbird species in the same area (nine eggs sampled from each of three species; $p < 0.001$), suggesting eggs to be good indicators for monitoring zinc. In a field study with passerines, Dauwe *et al*. (2005) found zinc concentrations in egg contents to be high compared with internal tissue concentrations in a sample of ten female great tits, although concentrations in the egg were poorly correlated with concentrations in both internal tissues and feathers. In a laboratory-based study, Williams *et al*. (1989b) found egg yolk zinc concentrations to be three times higher than controls in eggs produced by hens fed a diet containing 20000 mg/kg zinc for a period of four days.

Excess concentrations of zinc have been shown to have an adverse effect on egg production, and high dietary concentrations of zinc are routinely fed to laying domestic chickens (*Gallus* spp.) by poultry managers to force moulting and to reduce egg deposition, thus improving long-term egg production (Lu & Combs Jr., 1988; WHO, 2001b). In a study by Decuypere *et al*. (1988), laying hens fed 10000, 20000 and 30000 mg Zn/kg feed ceased laying completely. Palafox & Ho-A (1980) fed laying hens a diet containing 20000 mg Zn/kg feed and, although egg-laying did not completely cease, egg production was significantly lowered and eggs collected 14-28

days after the five day study period had reduced fertility and hatchability, with normal egg production, fertility and hatchability resuming during weeks 4-12, post-treatment. A significant reduction in body weight and egg production was observed in Japanese quail hens fed a diet containing 15000 mg/kg zinc for seven days, with thinner eggshells, reduced eggshell breaking strength and near-zero egg production by day three of treatment (Hussein *et al.*, 1988).

In terms of embryotoxicity, Birge and Roberts (1976) report survival rates of embryos of domestic chicken (*Gallus* spp.) eggs treated by yolk injection with a number of metals at various concentrations. Zinc injection to the egg resulted in 17% mortality at the lowest dose of 0.001 mg/kg egg; at 1.0 mg/kg 51% mortality was observed, and of the surviving embryos a further 8% exhibited gross malformations. At a dose of 50 mg/kg no embryos survived.

5.1.3 Partitioning of metals in eggs

The partitioning of different metals between eggshells and egg contents (combined yolk and albumen) has been examined by a number of authors. In a study looking at metal concentrations in eggshells and contents of the yellow-breasted chat (*Icteria virens*) and willow flycatcher (*Empidonax traillii extimus*), Mora (2003) found selenium and zinc primarily in egg contents; arsenic, nickel, lead and vanadium were detected mainly in eggshells. Manganese concentrations were slightly higher in eggshells, and results for zinc were conflicting between the two different species, with higher concentrations measured in egg contents than in shells for the yellow-breasted chat, and higher concentrations in the eggshell than the contents for the willow flycatcher. In another study with the short-tailed albatross (*Phoebastria albatrus*) and black-footed albatross (*Phoebastria nigripes*), Ikemoto *et al*. (2005) found cadmium and lead mainly in eggshells, and copper, manganese, selenium, vanadium and zinc mainly in egg contents. Burger (1994) reports higher concentrations of lead and selenium in egg contents than in eggshell, with manganese concentrations being fairly evenly spilt between the two (although slightly higher in eggshell). Results for cadmium were conflicting between the two species examined, with concentrations in herring gull (*L. argentatus*) eggs higher in the eggshell than in the egg contents, whereas for roseate terns (*Sterna dougallii*) concentrations were higher in the egg contents. In all studies a proportion of most metals analysed for was found to accumulate in the eggshell. Data of eggshell:egg contents (combined yolk and albumen) ratios reported in these studies are summarised below in Table 5-12.

Table 5-12 Eggshell:egg contents ratios of metals in different bird species

Metal	Yellow-breasted chat (a)	Willow flycatcher (a)	Short-tailed albatross (b)	Black-footed albatross (b)	Black-crowned night heron (d)	Little egret (d)	Roseate tern (c)	Bridled tern (d)	Herring gull (c)	Black-tailed gull (e)	Audouin's gull (f)
Arsenic	8.4	5.2	-	-	-	-	-	0.22	-	-	-
Cadmium	-	-	18.6	13.9	-	6.0	0.5	1.0	5.0	0.62	-
Cobalt	-	-	-	-	9.59	12.7	-	15.3	-	17.2	-
Copper	1.9	1.2	0.16	0.17	0.18	0.24	-	0.32	-	0.13	0.83
Iron	-	-	-	-	-	-	-	-	-	-	-
Lead	2.4	3.6	11.0	3.9	4.29	10.86	0.5	6.0	0.1	2.65	-
Manganese	1.1	2.1	1.4	0.62	0.60	2.79	1.02	0.42	1.5	0.62	0.17
Nickel	16.4	26.0	-	-	-	-	-	-	-	-	-
Selenium	0.17	0.35	0.03	0.03	2.49	2.27	0.001	3.55	0.13	0.13	-
Vanadium	20.8	19.2	0.62	0.35	1.38	3.57	-	-	-	0.27	-
Zinc	0.18	1.2	0.09	0.05	0.14	0.19	-	0.05	-	0.01	0.11

(a) Mora, 2003
(b) Ikemoto *et al*., 2005
(c) Burger, 1994
(d) Lam *et al*., 2005
(e) Agusa *et al*., 2005
(f) Morera *et al*., 1997

Clearly the evidence for the partitioning of many metals between eggshells and egg contents is conflicting. In addition to differences in concentrations between eggshells and egg contents, different metals have been found to bind to different components of the egg contents, with selenium and mercury binding preferentially to albumen and yolk, respectively (Magat & Sell, 1979). However, no data are available regarding the partitioning of any other metals between yolk and albumen. Knowledge of the partitioning of metals within the egg contents would enable a more targeted approach to metal analysis in eggs; for example, if a metal is found entirely in the yolk analysis of homogenised egg contents will only serve to dilute the concentrations of these metals in the sample. This is particularly important when concentrations are very low, and in this case it would be more prudent to analyse the egg yolk rather than contents as a whole. Ideally, all egg components (separated yolk, albumen and shell) should be analysed separately to gain some insight into where metals are partitioned in the egg and to obtain accurate results for total concentrations in the egg.

5.2 Methods

Details of egg collection procedures in 2005 and 2006 are provided in Section 2.2.1.

Samples of egg shell, yolk and albumen were analysed individually for arsenic, cadmium, cobalt, copper, iron, lead, manganese, nickel, selenium, vanadium and zinc.

Eggs were surface-cleaned with Milli-Q® water in order to remove any excrement, mud, plant debris etc., and measurements of the maximum length, width and weight of the whole egg were

recorded. Eggs were then opened using a clean, sterile scalpel, separated, the wet-weight of the individual egg components (yolk, albumen and shell) was recorded, and the eggshell thickness measured (See Section 2.2 for further details). Samples were then dried at 60ºC to a constant weight, dry weights were taken and the water content of each individual sample calculated. Each sample was ground and homogenised using a pestle and mortar, and approximately 0.2 g of each sample weighed out into a microwave digestion vessel as described below (exact weights recorded), with 10 ml of concentrated nitric acid added to each vessel.

Contamination was minimised at all stages by acid-washing all glassware prior to rinsing with Milli-Q® water, and rinsing any metal equipment with ultra high purity (BDH Chemicals ARISTAR®) grade acetone before bringing it into contact with samples. In addition, all procedures were carried out in a laminar-flow cabinet .

A CEM Microwave Accelerated Reaction System 5 (MARS 5^{TM}) was used to digest all of the samples prior to metals analysis. XP-1500 digestion vessels were used, which consist of a fluoropolymer liner (TFM®, a thermally resistant form of Teflon®) and a perfluoroalkoxy (PFA) resin cap. Vessels were assembled according to the manufacturer's instructions, placed in the microwave and heated over a 20 minute period until the temperature reached 230ºC. This temperature was then maintained for 10 minutes, after which the vessels were allowed to cool. Once cooled to a temperature below 50ºC, vessels were vented in a fume cupboard to release any remaining pressure, and a 1 ml aliquot of the digestate added to 9 ml of Milli-Q® water in a separate container (making a 10% nitric acid solution). This digestion technique is based on United States Environmental Protection Agency (USEPA) Method 3051. The method liberates virtually all bioavailable metals from their matrix and is an established technique for environmental samples (USEPA, 1996).

Once digested, samples were further diluted to approximately 2% nitric acid solution, prior to analysis by ICP-MS, which is a sensitive analytical technique capable of multi-elemental analysis. With each batch of samples determined, a calibration graph was constructed to check for linearity and to calculate concentrations in the samples. The calibration samples contained all of the elements of interest. In addition to a calibration, blank samples of 2% nitric acid were analysed to assess contamination. Measured samples were blank-corrected to provide reportable data.

To determine if any bias exists in the environmental media compared with the Milli-Q® water calibrant samples (used to construct a calibration curve and hence calculate concentrations),

spike-and-recovery tests were carried out for each different matrix (i.e. eggshell, yolk and albumen), for all the elements analysed for. This was achieved by spiking samples with varying concentrations of metal standards and constructing individual calibration curves. If the slope of the standard addition is less or greater than that for deionised water, this suggests an interferent is present in the sample that is either enhancing or suppressing the signal from the ICP-MS compared with deionised water. Accordingly, all calculations of metal concentrations in the samples were calculated using slopes derived from calibrations established in their own biological matrix.

These spike-recovery trials enabled assessment of the losses/gains made during the sample preparation method. Trials were carried out for all the metals investigated in this study.

5.2.1 Results of initial trials

For each trial, domestic chicken (*Gallus* spp.) eggs were separated into yolk, albumen and shell. Each egg was carefully opened and the albumen separated into a glass beaker. The yolk was gently rolled on a clean sheet of laboratory paper in order to remove any excess albumen. The egg shell was rinsed carefully with Milli-Q® water in order to remove any residual albumen, leaving membranes intact. Yolk, albumen and shell samples were then divided roughly in half and placed in pre-weighed glass jars before obtaining the wet weight of the samples. One half of each component from each egg was spiked with standard solutions of metals, while the corresponding half was left unspiked as a control sample.

Egg samples were spiked with varying amounts of a multi-element standard (Fisher Scientific, WP-15) containing 100 mg/l arsenic, copper, iron, manganese, nickel, lead and zinc, 25 mg/l cadmium and selenium and 250 mg/l vanadium, in a matrix of 5% nitric acid. Egg 1 yolk and albumen were spiked with 0.1 ml of this standard, Egg 2 samples with 0.5 ml, Egg 3 samples with 1 ml, Egg 4 samples with 2.5 ml and Egg 5 samples with 5 ml.

Spiked samples were mixed thoroughly with a glass rod. All samples (spiked and unspiked) were then dried in an oven at 60ºC to constant mass, after which dry weights were recorded and the water content of each sample calculated. The dried contents of each jar were then ground with a pestle and mortar, and an accurately weighed portion (*ca*. 0.2g) of the dried, homogenised sample was transferred to a microwave extraction vessel, as described in Section 5.2. The samples were microwave digested following the procedure outlined in Section 5.2,

after which analysis was carried out by ICP-MS. Table 5-13 provides recovery data from these trials.

Table 5-13 Mean recoveries for metals in chicken (*Gallus* spp.) egg components

Metal	Egg component	Recovery range (%)	Mean recovery (%)
As	Yolk	78.2 - 92.5	88.2
	Albumen	62.5 - 94.8	85.1
	Shell	66.9 - 83.1	73.9
Cd	Yolk	83.0 - 107.7	96.2
	Albumen	63.3 - 97.1	87.5
	Shell	67.5 - 79.7	74.1
Co	Yolk	87.0 - 116.7	102.7
	Albumen	70.6 - 103.6	94.6
	Shell	76.0 - 99.3	86.1
Cu	Yolk	88.9 - 133.2	108.3
	Albumen	66.0 - 100.5	85.9
	Shell	70.5 - 93.6	79.9
Fe	Yolk	104.6 - 158.7	138.4
	Albumen	65.4 - 79.1	73.8
	Shell	60.0 - 129.8	82.5
Pb	Yolk	96.8 - 118.5	106.9
	Albumen	66.4 - 103.9	91.5
	Shell	77.3 - 93.3	85.1
Mn	Yolk	88.2 - 116.6	106.4
	Albumen	68.9 - 103.6	94.0
	Shell	73.1 - 90.9	82.9
Ni	Yolk	88.2 - 120.8	105.7
	Albumen	67.9 - 102.1	90.7
	Shell	69.9 - 109.6	87.6
Se	Yolk	77.8 - 111.7	93.1
	Albumen	59.4 - 93.7	78.6
	Shell	68.2 - 78.2	73.6
V	Yolk	91.1 - 111.0	103.9
	Albumen	67.5 - 102.6	92.2
	Shell	76.6 - 96.0	83.8
Zn	Yolk	50.3 - 130.0	100.6
	Albumen	72.5 - 127.9	83.2
	Shell	59.1 - 80.0	68.4

Recovery data were good for all metals, with between 73 and 109% average recovery. The only exception is iron in egg yolk, for which the average recovery was 138.4%. The concentration of iron measured in egg yolk was consistently higher than the concentration than the expected

concentration in the spiked sample. This may be due to some external contamination, or polyatomic interference (i.e. molecular ions in the sample matrix with the same mass/charge ratio as the analyte of interest) in the yolk leading to an artificial increase in the iron concentration measured. The most commonly reported interferents with iron analysis by ICP-MS are argon and calcium (May & Wiedmeyer, 1998; Segura *et al.*, 2003); interference due to argon oxide would arise as a result of argon gas in the ICP-MS and would therefore lead to an artificial increase in the iron concentrations for all samples. However, in this study the recoveries indicate an artificial increase in iron concentration measured only for yolk samples, and thus it is more likely that the polyatomic interference in this case is due to the high calcium content of egg yolk. As the recoveries for iron measured in yolk samples were fairly consistent at around 130-150% (with the exception of the lowest spiked sample), data could be adjusted accordingly to account for the over-estimation of the iron concentration.

Overall, the recovery data using the method outlined above suggests that this method is suitable for the analysis of the above metals in egg yolk, albumen and shell, although iron concentrations in yolk may be over-estimated. In order to avoid over- or under-estimates of the concentrations measured in the eggs using the method outlined above, all of the raw analytical results were corrected according to the recoveries in Table 5-13. For example, for arsenic in egg yolk the recovery data suggests that only 88.2% of the total concentration of arsenic in each sample will be recovered using this method. Thus the actual arsenic concentration in a yolk sample measured at 10 μg/g, for example, would be 10*(100/88.2) = 11.34 μg/g.

A full set of recovery data with individual concentrations of each metal in egg components is provided in Appendix C.

5.3 Results

5.3.1 Relationship between metal concentrations and egg characteristics

Pearson's correlation was carried out for the egg characteristics (as analysed in Chapter 2) and total metal concentration in the egg (the combined sum of the concentration measured in yolk, albumen and shell) to identify any relationships between total egg metal concentrations and egg characteristics.

Table 5-14 provides values for Pearson's correlation (r) between the total metal concentrations and characteristics of black-headed gull eggs.

Table 5-14 Pearson's correlations between total metal concentrations and characteristics of black-headed gull eggs for all sites studied, 2005 and 2006

Measurement	Total As conc.	Total Cd conc.	Total Co conc.	Total Cu conc.	Total Fe conc.	Total Pb conc.	Total Mn conc.	Total Ni conc.	Total Se conc.	Total V conc.	Total Zn conc.
Yolk wet weight	0.416	0.251	0.364	0.051	0.088	0.060	0.116	0.559	0.617	0.388	0.138
Albumen wet weight	0.626	0.716	**0.010**	0.663	**0.033**	**0.005**	0.337	**0.008**	0.236	0.964	0.456
Y:A ratio	0.822	0.619	**0.015**	0.433	**0.008**	0.353	0.922	**0.015**	0.420	0.643	0.192
Egg length	0.416	0.729	0.483	0.397	0.804	0.054	0.092	0.133	0.156	0.237	0.426
Egg width	0.104	0.484	0.122	0.923	0.188	0.139	0.294	0.408	0.660	0.776	0.444
Shell thickness	0.308	0.991	0.145	0.136	0.503	0.242	0.808	0.124	0.996	0.973	0.166
Shell index	0.640	0.360	0.675	0.796	0.087	0.589	0.640	0.289	0.441	0.084	0.511

The correlation matrix revealed a significant interaction between metal concentration and wet weight of albumen and yolk:albumen ratio for cobalt (Pearson's correlation r = 0.306, p = 0.010 for wet weight albumen and r = -0.292, p = 0.015 for yolk:albumen ratio), iron (r = 0.257, p = 0.033 for wet weight albumen and r = -0.316, p = 0.008 for yolk:albumen ratio) and nickel (r = 0.327, p = 0.010 for wet weight albumen and r = -0.293, p = 0.015 for yolk:albumen ratio). Concentrations of lead were also significantly correlated with wet weight of albumen (r = 0.334, p = 0.005), but not with yolk:albumen ratio (r = -0.133, p = 0.353). No other significant interactions between metal concentration and egg parameters were found.

5.3.2 Total metal concentrations in the egg

Figures 5-1 to 5-11 show mean concentrations (with standard error bars) of each of the metals analysed in eggs from each of the sample sets. N = 69: n = 20, 19, 20 and 10 for Lymington Early, Lymington Late, Poole and Raby sample sets, respectively.

For comparison, a mean concentration calculated from values reported in the literature for Laridae on a global scale is presented (for actual concentrations reported in the literature, see Section 5.1.2, Tables 5-2 to 5-11).

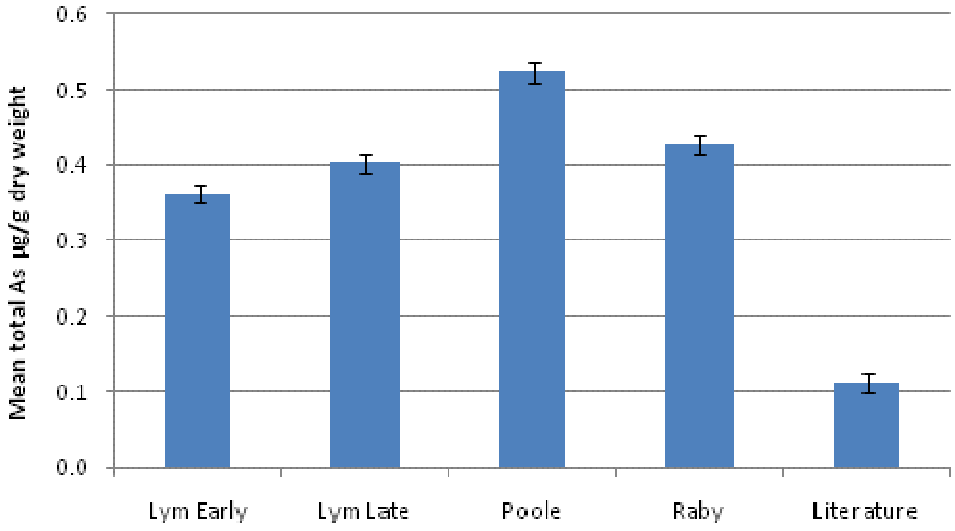


Figure 5-1 **Mean total arsenic concentrations (µg/g dry weight), ± standard error, in black-headed gull eggs from sampling sites in this study (N = 69), compared with literature data for gull eggs worldwide (N = 2)**

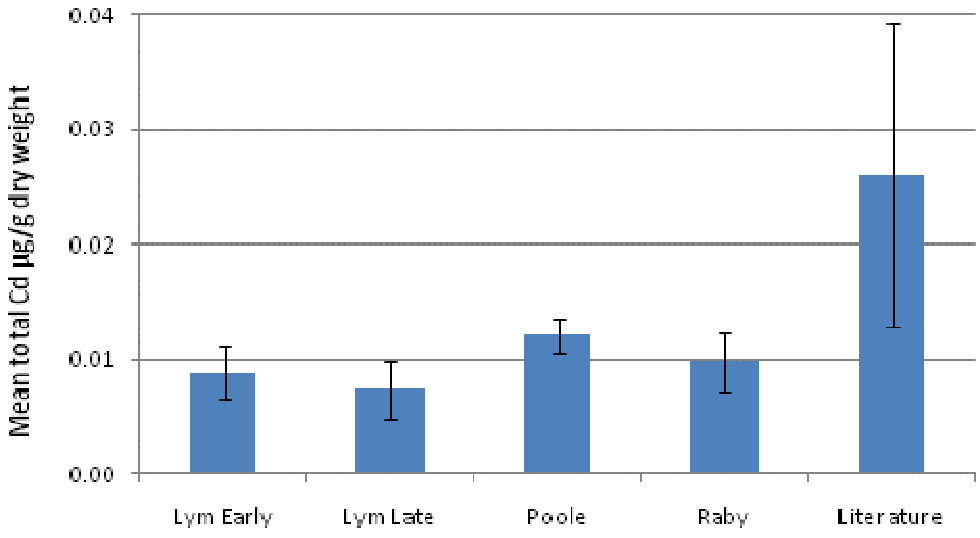


Figure 5-2 **Mean total cadmium concentrations (µg/g dry weight), ± standard error, in black-headed gull eggs from sampling sites in this study (N = 69), compared with literature data for gull eggs worldwide (N = 4)**

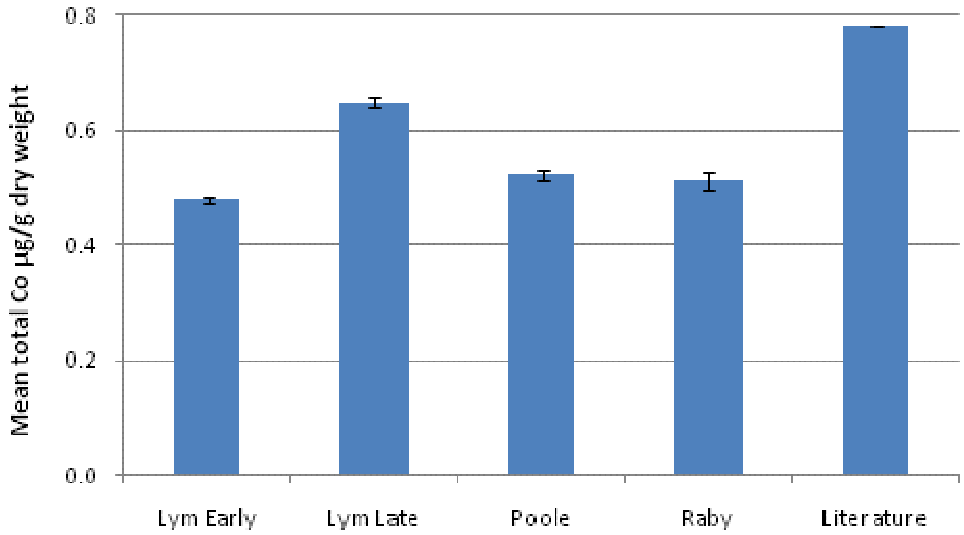


Figure 5-3 **Mean total cobalt concentrations (µg/g dry weight), ± standard error, in black-headed gull eggs from sampling sites in this study (N = 69), compared with literature data for gull eggs worldwide (N = 1)**

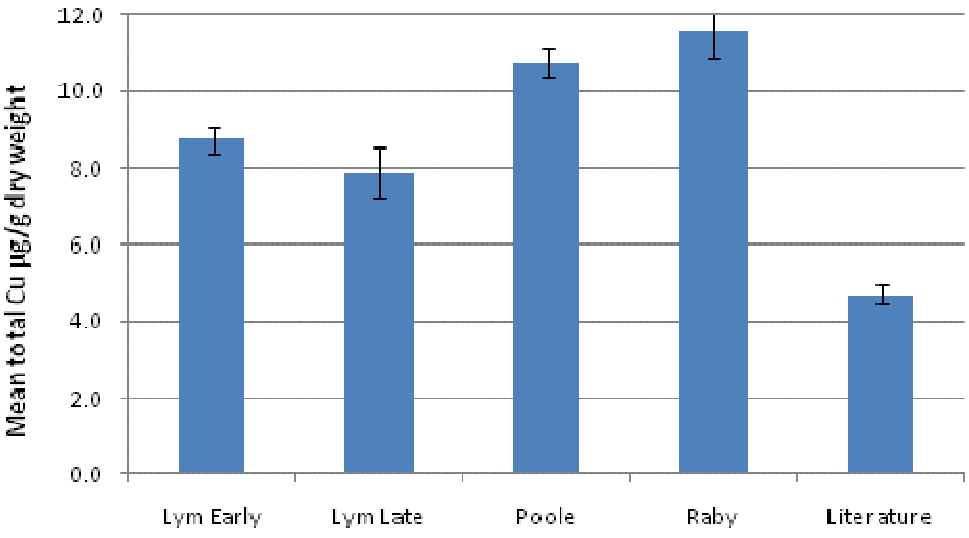


Figure 5-4 **Mean total copper concentrations (µg/g dry weight), ± standard error, in black-headed gull eggs from sampling sites in this study (N = 69), compared with literature data for gull eggs worldwide (N = 2)**

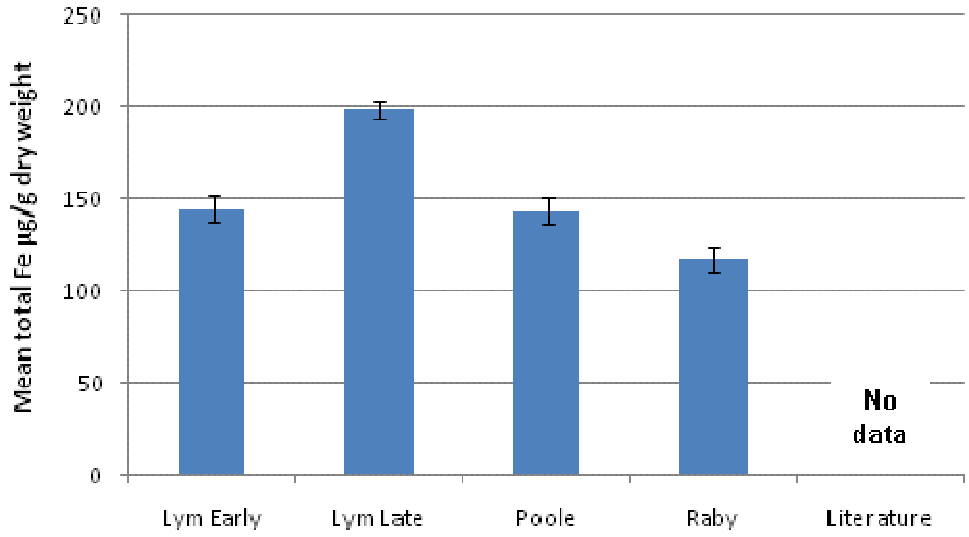


Figure 5-5 **Mean total iron concentrations (µg/g dry weight), ± standard error, in black-headed gull eggs from sampling sites in this study (N = 69)**

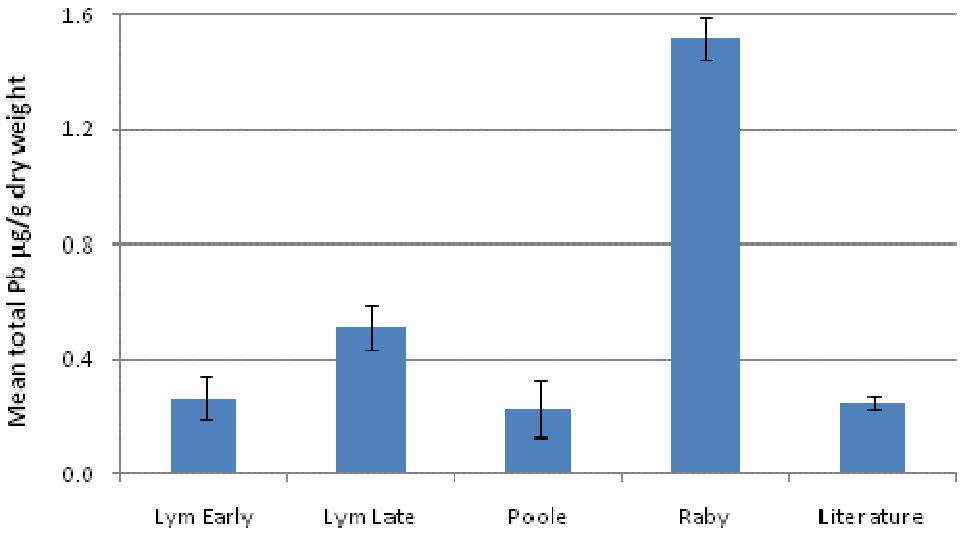


Figure 5-6 **Mean total lead concentrations (µg/g dry weight), ± standard error, in black-headed gull eggs from sampling sites in this study (N = 69), compared with literature data for gull eggs worldwide (N = 2)**

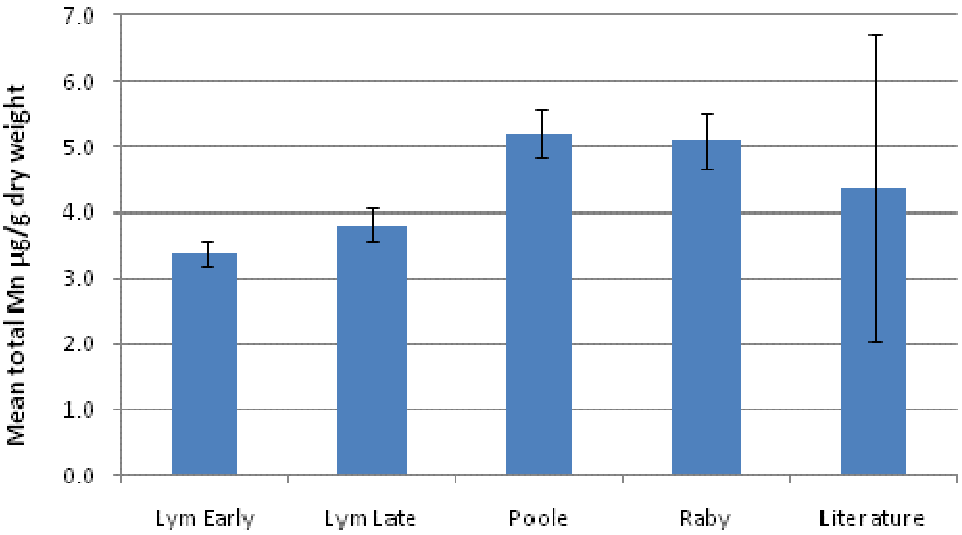


Figure 5-7 **Mean total manganese concentrations (µg/g dry weight), ± standard error, in black-headed gull eggs from sampling sites in this study (N = 69), compared with literature data for gull eggs worldwide (N = 5)**

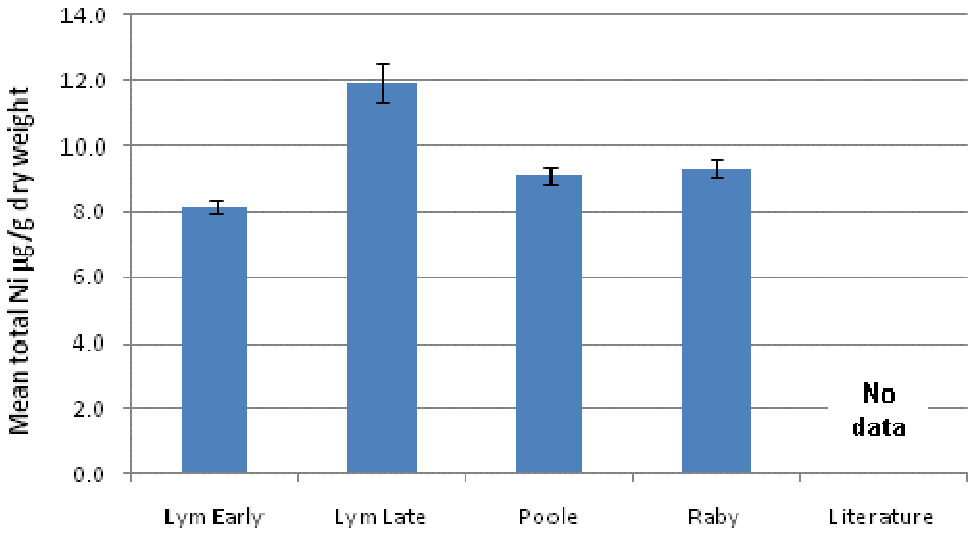


Figure 5-8 **Mean total nickel concentrations (µg/g dry weight), ± standard error, in black-headed gull eggs from sampling sites in this study (N = 69)**

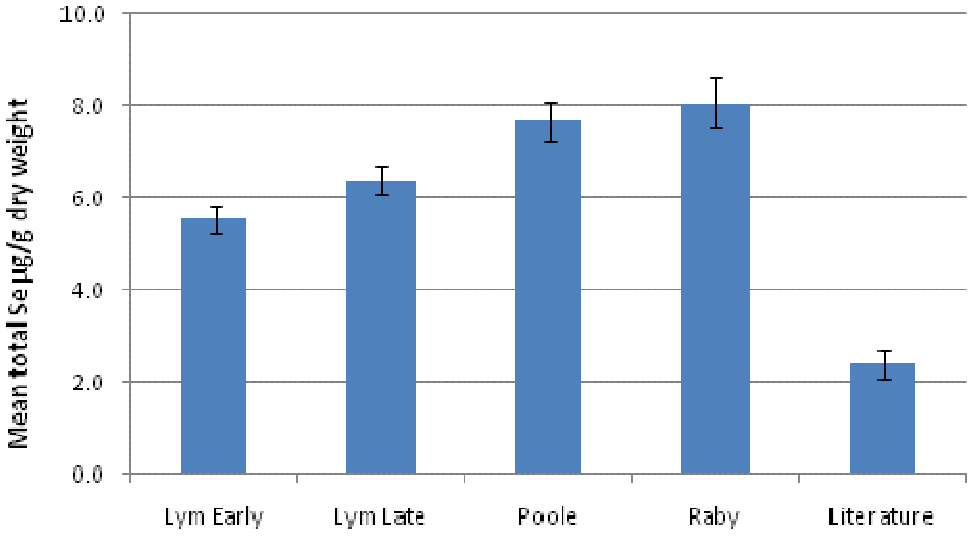


Figure 5-9 **Mean total selenium concentrations (µg/g dry weight), ± standard error, in black-headed gull eggs from sampling sites in this study (N = 69), compared with literature data for gull eggs worldwide (N = 8)**

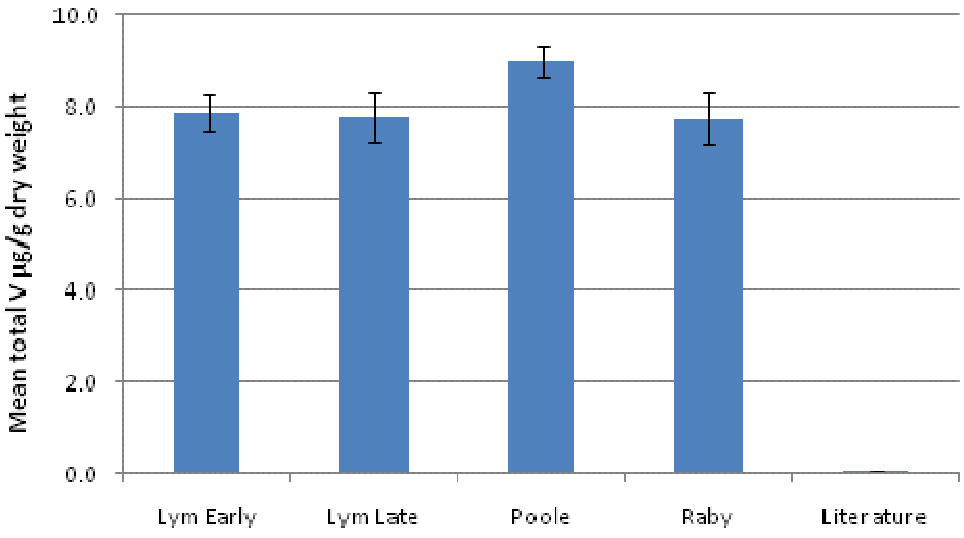


Figure 5-10 **Mean total vanadium concentrations (µg/g dry weight), ± standard error, in black-headed gull eggs from sampling sites in this study (N = 69), compared with literature data for gull eggs worldwide (N = 1)**

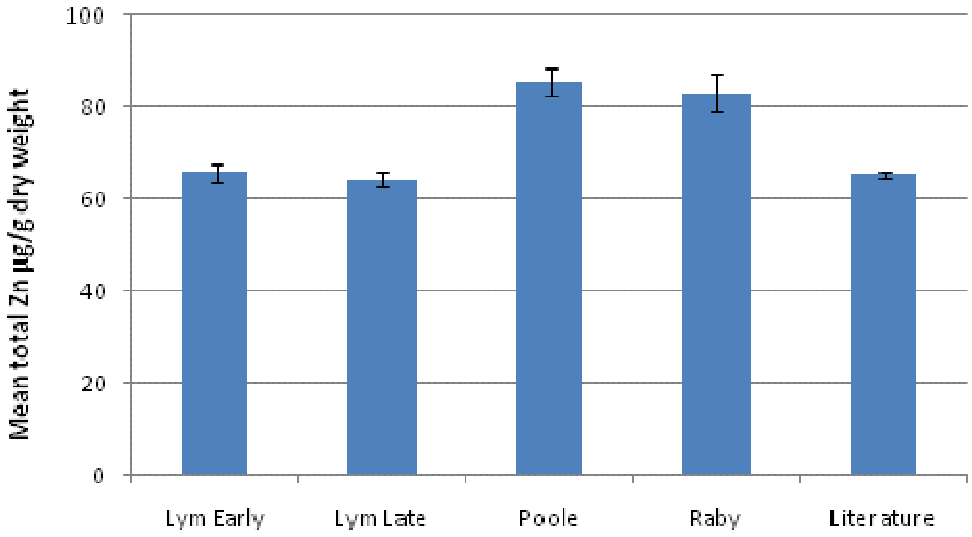


Figure 5-11 **Mean total zinc concentrations (µg/g dry weight), ± standard error, in black-headed gull eggs from sampling sites in this study (N = 69), compared with literature data for gull eggs worldwide (N = 2)**

Statistical analysis

To check that data were normally distributed, a K-S test was carried out; this test revealed that data were normally distributed for arsenic, copper, iron, manganese, selenium, vanadium and zinc. The distributions of cadmium (K-S statistic $D = 0.177$, $p < 0.001$), cobalt ($D = 0.130$, $p = 0.05$), lead ($D = 0.220$, $p < 0.001$) and nickel ($D = 0.203$, $p < 0.001$) were not normal. Various transformations were tried in order to attempt to normalise the data, including logarithmic, reciprocal, squared, cubed, square root, inverse square root, cube root and inverse cube root. Logarithmic transformation produced a normalised distribution of the lead data (K-S statistic $D = 0.099$; $p = 0.089$). However, the data for cadmium, cobalt and nickel could not be normalised with any of the transformations attempted. Data for cadmium, cobalt and nickel do not therefore meet the requirements for parametric tests, and must therefore be explored via non-parametric tests. Variance was examined using Levene's test for equality of variance.

In order to assess whether any significant differences existed between the site sample sets (Lymington Early, Lymington Late, Poole and Raby) in terms of metal concentrations, a one-way ANOVA was carried out. A summary of the ANOVA data is provided in Table 5-15. ANOVA data provided in the table is based on the raw, untransformed data for all metals except lead, data for which was log transformed prior to running ANOVA, and then calculated back to the original scale for the purposes of reporting. For cadmium, cobalt and nickel a non-parametric Kruskal-Wallis test was used as an alternative to one-way ANOVA (Townend, 2002). The test requires the distributions of the values in the datasets being tested to be the same shape, although not necessarily normal. As the datasets for cadmium, cobalt and nickel are all similarly skewed to the left, the requirements for this test are met.

Table 5-15 provides a summary of the results from these tests. The results show that there are significant differences between sample sets for the total egg concentrations of arsenic, cadmium, cobalt, copper, iron, lead, selenium and zinc. There are no significant differences between sample sets for the total egg concentrations of vanadium.

Table 5-15 Test results for differences in total metal concentrations in black-headed gull eggs between sites, 2005-2006

Measurement	n	F value	p	Significance
Arsenic				
Lym Early	20	3.864	0.013	*
Lym Late	19			
Poole	20			
Raby	10			
Cadmium				
Lym Early	20	Chi sqare 12.425	0.006	**
Lym Late	19			
Poole	20			
Raby	10			
Cobalt				
Lym Early	20	Chi sqare 48.844	<0.001	**
Lym Late	19			
Poole	20			
Raby	10			
Copper				
Lym Early	20	10.196	<0.005	**
Lym Late	19			
Poole	20			
Raby	10			
Iron				
Lym Early	20	25.592	<0.005	**
Lym Late	19			
Poole	20			
Raby	10			
Lead				
Lym Early	20	14.681	<0.005	**
Lym Late	19			
Poole	20			
Raby	10			
Manganese				
Lym Early	20	9.763	<0.005	**
Lym Late	19			
Poole	20			
Raby	10			
Nickel				
Lym Early	20	Chi square 38.416	<0.001	**
Lym Late	19			
Poole	20			
Raby	10			
Selenium				
Lym Early	20	8.924	<0.005	**
Lym Late	19			
Poole	20			
Raby	10			
Vanadium				
Lym Early	20	1.916	0.136	NS
Lym Late	19			
Poole	20			
Raby	10			
Zinc				
Lym Early	20	20.759	<0.005	**
Lym Late	19			
Poole	20			
Raby	10			

Bold indicates a significant difference between sample sets for that variable;
* = significant ($p \leq 0.05$);
** = highly significant ($p \leq 0.001$);
NS = not significant ($p > 0.05$).

Data are for one-way ANOVA with the exception of highlighted areas , which indicate results from non-parametric Kruskal-Wallis test.

Having established significant differences between sample sites, post-hoc tests were used to identify which groups were significantly different from one another for each of the metals. A Tukey test was used to examine the differences between groups for the metals. However, the Tukey test is a parametric post-hoc test and is therefore unsuitable for examination of the metals which are not normally distributed; thus cadmium, cobalt and nickel are not included in the Tukey test.

A summary of the data from the Tukey test is provided in Table 5-16.

Table 5-16 Tukey test results for differences between sites: total metal concentrations in black-headed gull eggs, 2005-2006

Metal	Significant site differences	n	S.E.	p	Significance level
Arsenic	Poole > Lym Early	40	0.049	0.009	**
Copper	Poole > Lym Early	40	0.663	0.020	*
	Poole > Lym Late	39	0.672	<0.001	**
	Raby > Lym Early	30	0.813	0.005	**
	Raby > Lym Late	29	0.820	<0.001	**
Iron	Lym Late > Lym Early	39	8.605	<0.001	**
	Lym Late > Poole	39	8.605	<0.001	**
	Lym Late > Raby	29	10.494	<0.001	**
Lead	Lym Late > Poole	39	0.113	0.012	*
	Raby > Lym Early	30	0.136	<0.001	**
	Raby > Lym Late	29	0.138	0.006	**
	Raby > Poole	30	0.136	<0.005	**
Manganese	Poole > Lym Early	40	0.389	<0.001	**
	Poole > Lym Late	39	0.394	0.005	**
	Raby > Lym Early	30	0.476	0.003	**
	Raby > Lym Late	29	0.480	0.044	*
Selenium	Poole > Lym Early	40	0.495	<0.001	**
	Raby > Lym Early	30	0.607	0.001	**
	Raby > Lym Late	29	0.612	0.040	*
Vanadium	None	-	-	-	-
Zinc	Poole > Lym Early	40	3.251	<0.001	**
	Poole > Lym Late	39	3.294	<0.001	**
	Raby > Lym Early	30	3.982	<0.001	**
	Raby > Lym Late	29	4.016	<0.001	**

* = significant ($p \leq 0.05$); ** = highly significant ($p \leq 0.01$). S.E. = standard error.

The results show that the eggs from the Poole colony have significantly higher mean concentrations of arsenic, copper, manganese, selenium and zinc than those from the Lymington

Early sample set (i.e. those eggs collected before the period of commercial egg harvesting commenced). The Poole eggs also showed higher mean concentrations than the Lymington Late eggs (i.e. those collected at the end of the commercial egg harvesting period) for copper, manganese and zinc.

The Lymington Late eggs exhibited higher mean concentrations of iron than any of the other three sample sets, and higher lead concentrations than the Poole eggs. Eggs from the Raby site had a significantly higher concentration of lead than the eggs from any of the other sample sets. The Raby eggs also had higher concentrations of copper, selenium and zinc than either Lymington Early or Lymington Late eggs; however, there was no significant difference between the Raby and the Poole sites for these metals.

Cadmium, cobalt and nickel, the data not being normally distributed, were examined with a non-parametric post-hoc test. A series of Mann-Whitney tests were used to examine the differences between groups for these metals. This method requires conducting separate tests comparing each group with each of the other groups, which, in this case, requires six different Mann-Whitney tests to examine the six different group combinations. However, the use of multiple tests will inflate the Type I error rate (i.e. the chance of falsely rejecting the null hypothesis), and thus an adjustment of some kind should be made to ensure that the Type I errors don't amount to more than 0.05 (Field, 2005). The method employed to do this the Bonferroni correction, where the critical value of 0.05 is divided by the number of tests conducted (Field, 2005). In this case, six tests were conducted and the significance level is therefore taken as 0.05/6, i.e. 0.0083. Therefore, in order for a result to be considered significant, the p-value generated by the Mann-Whitney test must be ≤ 0.0083. The significant differences between groups highlighted by these tests are provided in Table 5-17.

Table 5-17 Significant Mann-Whitney test results for differences in total metal concentrations in black-headed gull eggs between sites, 2005-2006: Cd, Co and Ni

Metal	Significant site differences	n	Mann-Whitney U	P
Cadmium	Poole > Lym Late	39	90.0	0.004
Cobalt	Lym Late > Lym Early	39	0.00	<0.001
	Lym Late > Poole	39	1.00	<0.001
	Lym Late > Raby	29	1.00	0.001
	Poole > Lym Early	40	57.5	<0.001
Nickel	Lym Late > Lym Early	39	9.00	<0.001
	Lym Late > Poole	39	48.0	<0.001
	Lym Late > Raby	29	33.0	0.003
	Poole > Lym Early	40	69.0	<0.001
	Raby > Lym Early	30	29.0	0.001

Note: significant result $P \leq 0.0083$.

The results from the Mann-Whitney tests show that the Lymington Late eggs have significantly higher mean concentrations than any of the other sample groups for both cobalt and nickel. Poole eggs have significantly higher mean concentrations than the Lymington Early eggs for cobalt and nickel, and significantly higher mean concentrations of cadmium than Lymington Late eggs. Raby eggs also had significantly higher mean concentrations of nickel than the Lymington Early eggs.

5.3.3 Metal partitioning in the egg

Concentrations of heavy metals in each of the different egg components (yolk, albumen and shell) are shown in Figures 5-12 to 5-22. Data in the following figures were derived using combined data from all of the sites to provide a mean metal concentration in each egg component. Data from all sites were pooled to increase the sample size for statistical analysis, and to provide an overview of the general partitioning of each metal in the egg.

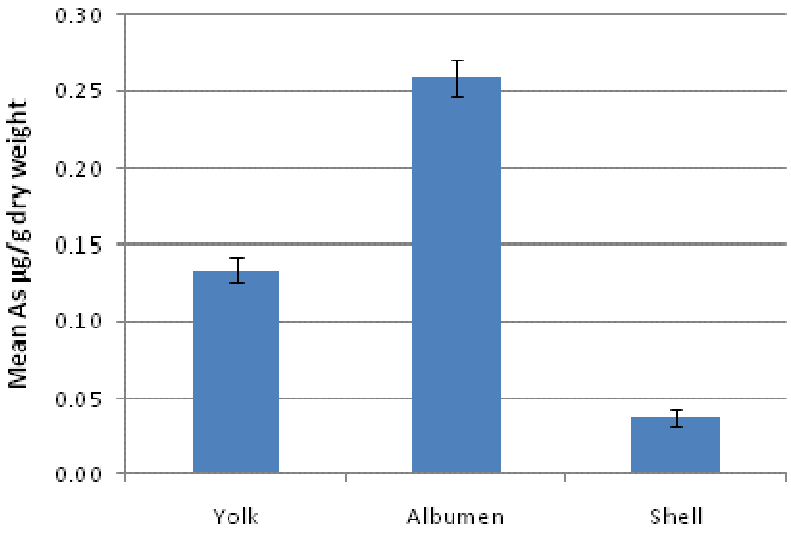


Figure 5-12 Mean arsenic concentrations (µg/g dry weight), ± standard error, in components of black-headed gull eggs, 2005-2006 (N = 69)

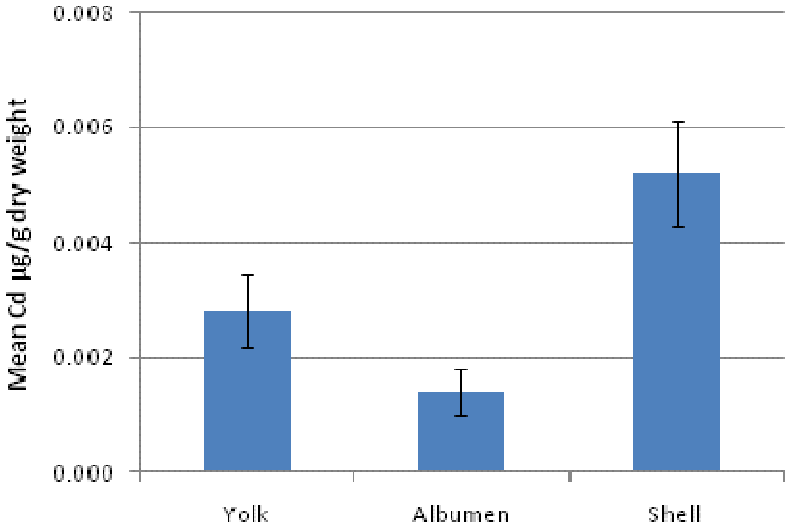


Figure 5-13 Mean cadmium concentrations (µg/g dry weight), ± standard error, in components of black-headed gull eggs, 2005-2006 (N = 69)

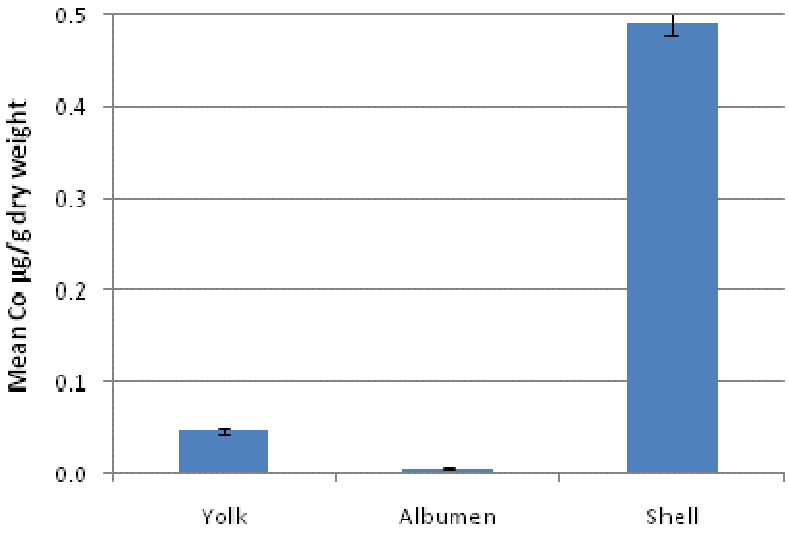


Figure 5-14 Mean cobalt concentrations (µg/g dry weight), ± standard error, in components of black-headed gull eggs, 2005-2006 (N = 69)

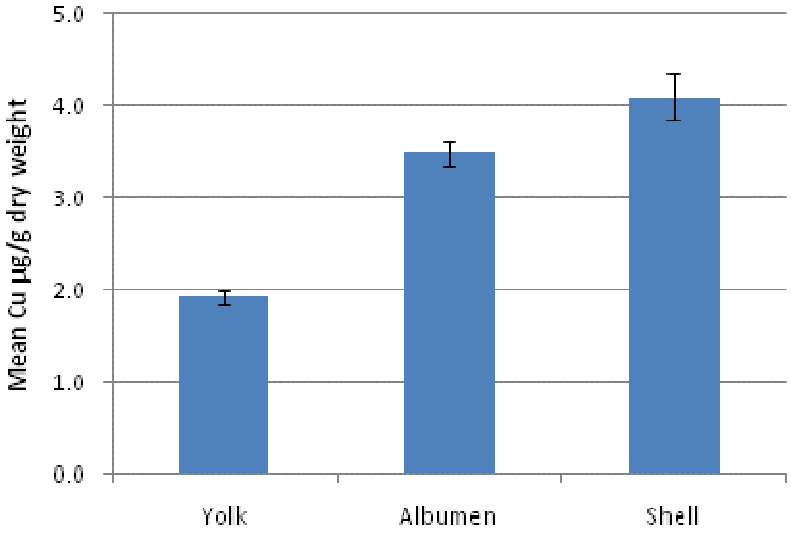


Figure 5-15 Mean copper concentrations (µg/g dry weight), ± standard error, in components of black-headed gull eggs, 2005-2006 (N = 69)

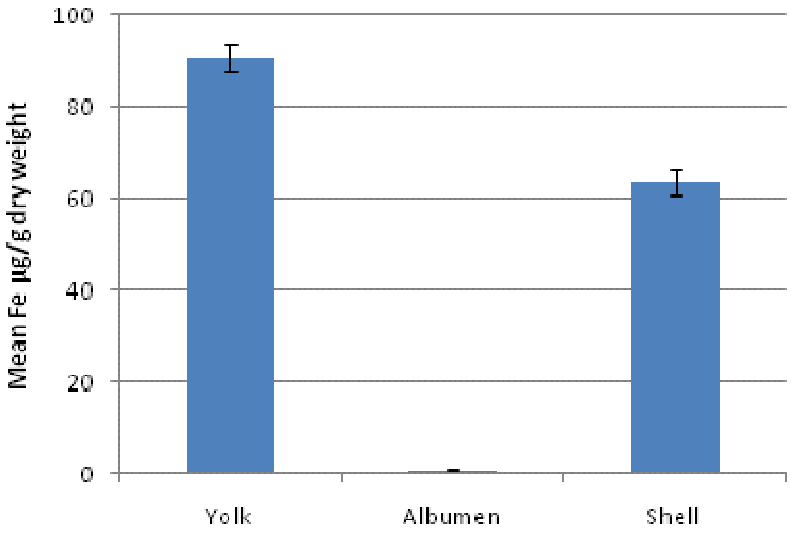


Figure 5-16 Mean iron concentrations (µg/g dry weight), ± standard error, in components of black-headed gull eggs, 2005-2006 (N = 69)

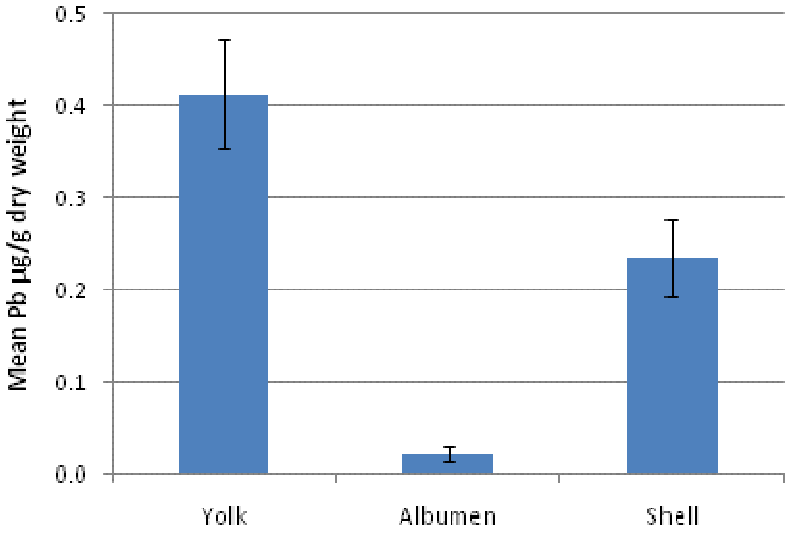


Figure 5-17 Mean lead concentrations (µg/g dry weight), ± standard error, in components of black-headed gull eggs, 2005-2006 (N = 69)

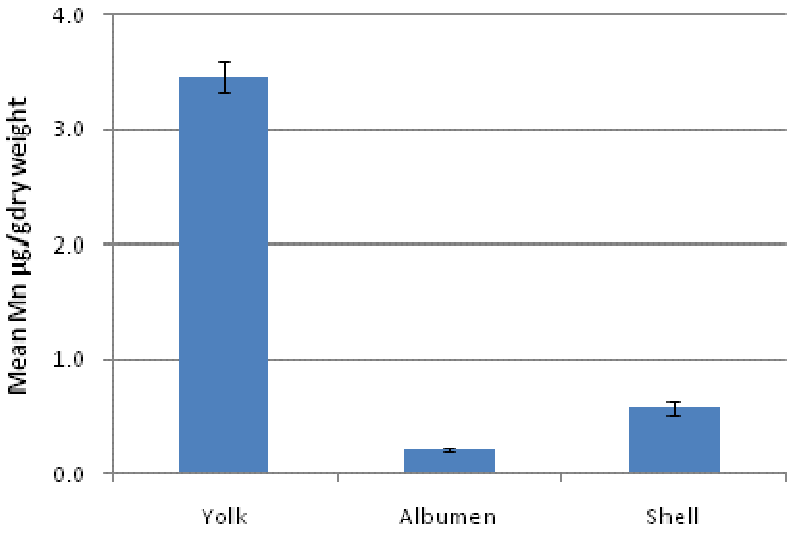


Figure 5-18 Mean manganese concentrations (µg/g dry weight), ± standard error, in components of black-headed gull eggs, 2005-2006 (N = 69)

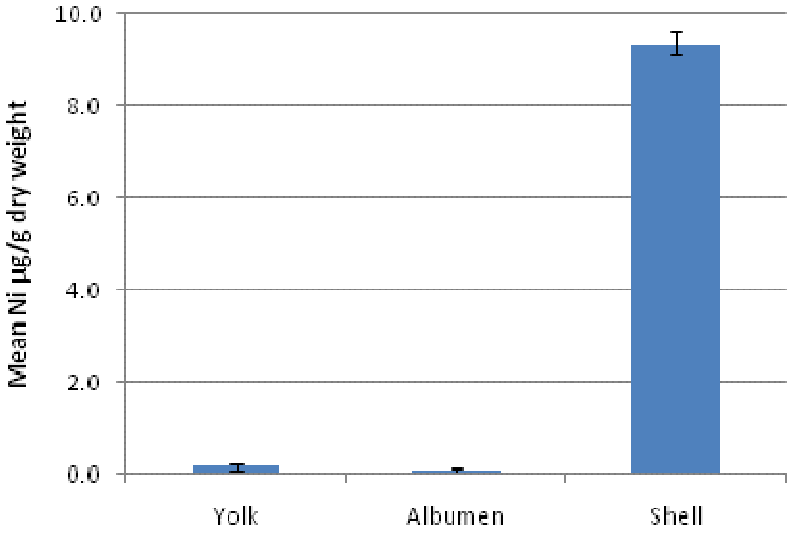


Figure 5-19 Mean nickel concentrations (µg/g dry weight), ± standard error, in components of black-headed gull eggs, 2005-2006 (N = 69)

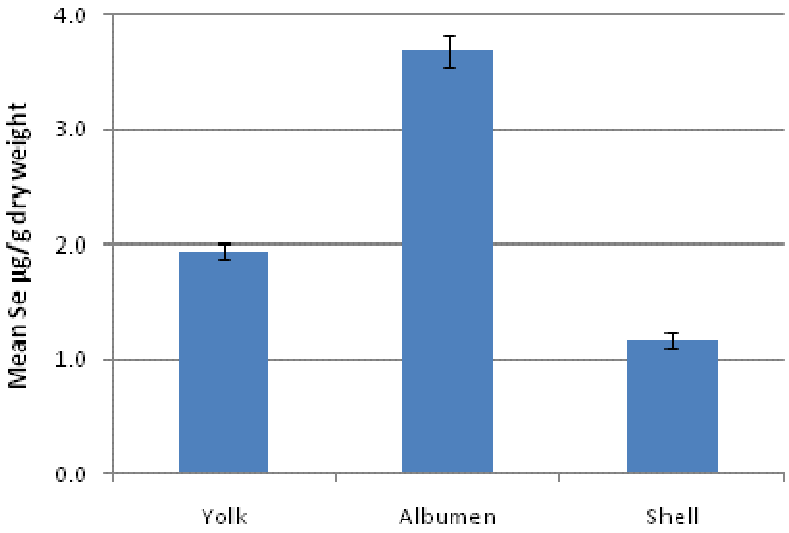


Figure 5-20 Mean selenium concentrations (µg/g dry weight), ± standard error, in components of black-headed gull eggs, 2005-2006 (N = 69)

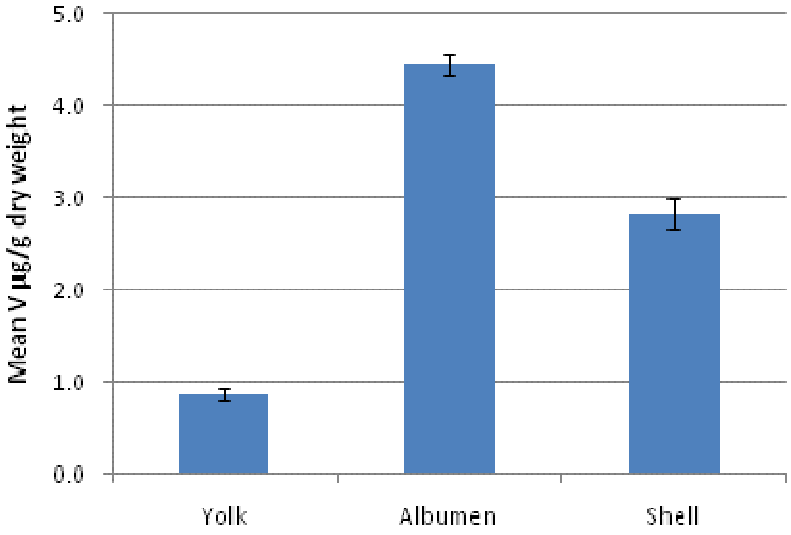


Figure 5-21 Mean vanadium concentrations (µg/g dry weight), ± standard error, in components of black-headed gull eggs, 2005-2006 (N = 69)

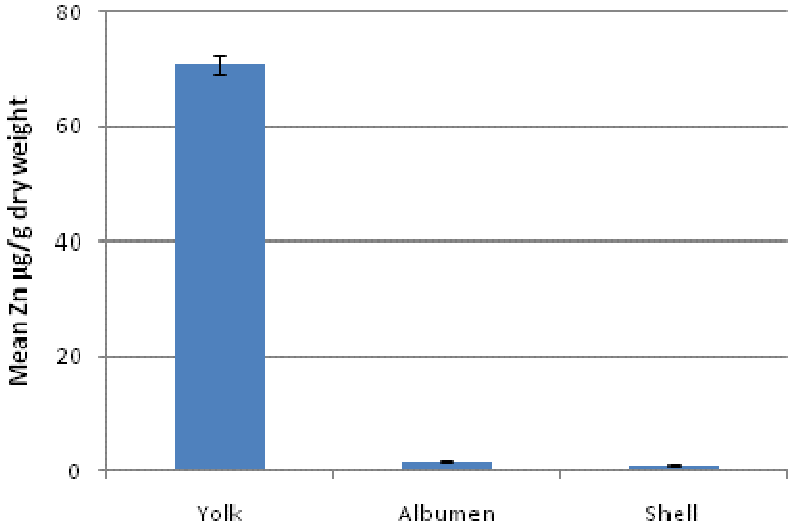


Figure 5-22 Mean zinc concentrations (µg/g dry weight), ± standard error, in components of black-headed gull eggs, 2005-2006 (N = 69)

Statistical analysis

A K-S test was carried out to check that data were normally distributed; this test revealed that data were normally distributed for copper, but not for any other metal. Various transformations were attempted to try to normalise the data (as detailed in Section 5.3.2); however, none of the data for any of the metals could be normalised with any of the transformations attempted. Data for arsenic, cadmium, cobalt, iron, manganese, nickel, lead, selenium, vanadium and zinc do not therefore meet the requirements for parametric tests, and must therefore be explored via non-parametric tests. Variance was examined using Levene's test for equality of variance.

In order to assess whether any significant differences existed between the sample sets in terms of copper concentrations, a one-way ANOVA was carried out. ANOVA revealed that there was a significant difference between the egg components for copper ($F = 40.81$; $p < 0.001$; $n = 69$). Note: ANOVA also suggested a significant difference between egg components for concentrations of all other metals ($p \leq 0.001$ for all), although these results cannot be considered reliable as the criteria for parametric testing were not met.

As the datasets for arsenic, cadmium, cobalt, iron, lead, manganese, nickel, selenium, vanadium and zinc are of similar shape, all being skewed to the left, the Kruskal-Wallis test was considered an appropriate non-parametric alternative to ANOVA for examining this data. Table 5-18 provides a summary of the data from the Kruskal-Wallis test.

Table 5-18 Kruskal-Wallis test results for differences in metal concentrations in black-headed gull eggs between yolk, albumen and shell, 2005-2006

	Chi-square	p	Significance
Arsenic	129.76	<0.001	**
Cadmium	26.60	<0.001	**
Cobalt	177.48	<0.001	**
Iron	155.46	<0.001	**
Lead	85.76	<0.001	**
Manganese	148.79	<0.001	**
Nickel	148.11	<0.001	**
Selenium	147.09	<0.001	**
Vanadium	142.09	<0.001	**
Zinc	140.65	<0.001	**

** = highly significant ($p \leq 0.001$); 0.05); n = 69.

The Kruskal-Wallis test confirms the suggestions made by the ANOVA that there are significant differences between the egg components for all metals analysed.

Having established significant differences between sample sites, post-hoc tests were used to identify which groups were significantly different from one another for each of the metals. A Tukey test was used to examine the differences between egg components for copper, the results of which, combined with Figures 5-12 to 5-22, show that the concentrations of copper were significantly higher in albumen than in yolk ($p < 0.001$, n = 69), and significantly higher in shell than yolk ($p < 0.001$, n = 69) and albumen (p 0.041, n = 69).

As the Tukey test is a parametric post-hoc test it is unsuitable for examination of the metals which are not normally distributed, and a non-parametric post-hoc test must be used for arsenic, cadmium, cobalt, iron, lead, manganese, nickel, selenium, vanadium and zinc. A series of Mann-Whitney tests were used to examine the differences in the concentrations of these metals between egg components. As described above (Section 5.3.2), conducting a series of tests necessitates an adjustment to the significance level; in this case, three tests were conducted and the significance level is therefore taken as 0.05/3, i.e. 0.017, and a p-value generated by of ≤ 0.017 therefore indicates a significant result. The significant differences between groups highlighted by these tests are provided in Table 5-19.

Table 5-19 Significant Mann-Whitney test results for differences in metal concentrations in black-headed gull eggs between yolk, albumen and shell, 2005-2006

Metal	Significant difference	Mann-Whitney U	p
Arsenic	Albumen > Yolk	736.0	<0.001
	Yolk > Shell	639.0	<0.001
	Albumen > Shell	74.0	<0.001
Cadmium	Shell > Yolk	1656.0	0.001
	Shell > Albumen	1245.5	<0.001
Cobalt	Yolk > Albumen	43.0	<0.001
	Shell > Yolk	69.0	<0.001
	Shell > Albumen	20.0	<0.001
Iron	Yolk > Albumen	0.0	<0.001
	Yolk > Shell	1016.0	<0.001
	Shell > Albumen	0.0	<0.001
Lead	Yolk > Albumen	193.0	<0.001
	Yolk > Shell	1464.0	<0.001
	Shell > Albumen	1275.5	<0.001
Manganese	Yolk > Albumen	0.0	<0.001
	Yolk > Shell	41.0	<0.001
	Shell > Albumen	1093.5	<0.001
Nickel	Yolk > Albumen	1376.5	<0.001
	Shell > Yolk	0.0	<0.001
	Shell > Albumen	0.0	<0.001
Selenium	Albumen > Yolk	288.0	<0.001
	Yolk > Shell	570.0	<0.001
	Albumen > Shell	72.0	<0.001
Vanadium	Albumen > Yolk	4.0	<0.001
	Shell > Yolk	413.0	<0.001
	Albumen > Shell	731.5	<0.001
Zinc	Yolk > Albumen	0.0	<0.001
	Yolk > Shell	0.0	<0.001

Note: significant result $P \leq 0.017$.

The results of the Mann-Whitney tests, combined with Figures 5-12 to 5-22, show that arsenic is consistently found at the highest concentrations in the albumen, which contains significantly higher concentrations than both the yolk and the shell. Concentrations of arsenic in the yolk are around 50% of those in the albumen, and shell concentrations are significantly lower in the shell than either yolk or albumen. Cadmium concentrations were low in all samples (Figure 5-13; all <0.01 μg/g dry weight), and in many samples cadmium was below the limit of detection (<0.001 μg/g). As a result, the standard errors for the mean cadmium concentration data are very high in the majority of cases and, although the results of the Mann-Whitney tests show cadmium concentrations to be significantly higher in the shell than either the yolk or albumen, the data should be treated with caution on account of the large margin of error associated. Both cobalt and nickel were detected mainly in the eggshell, with concentrations significantly higher than in either the yolk or albumen for both. The post-hoc tests also show that the yolk contains significantly higher concentrations of both cobalt and nickel than the albumen. However, it should be noted that for nickel the concentrations in the yolk and albumen were largely undetectable and thus the standard errors associated with this data are very high. Iron, lead, manganese and zinc were all partitioned mainly in the yolk, with significantly higher concentrations found in the yolk than both albumen and shell. Iron, lead and manganese concentrations were also significantly higher in shell than in albumen. Selenium concentrations were highest in the egg contents, with significantly higher concentrations in the albumen and yolk than in the shell; in terms of partitioning between the egg contents, selenium was found at significantly higher concentrations in the albumen than in the yolk. Concentrations of vanadium were significantly higher in the albumen than either yolk or shell, and significantly higher in the shell than the yolk.

Table 5-20 provides ratios for the partitioning of the metals analysed in this study between yolk and albumen. Data from all the sites in this study were pooled in order to provide an overview of the partitioning of metals in the contents of black-headed gull eggs.

Table 5-20 Yolk:albumen ratios of metals in black-headed gull eggs (combined data for all sites, 2005 and 2006)

Metal	Mean yolk conc. µg/g	Mean albumen conc. µg/g	Yolk/albumen ratio
Arsenic	0.118	0.221	**0.53**
Cadmium	0.002	0.001	**2.00**
Cobalt	0.048	0.006	**8.00**
Copper	2.072	3.083	**0.67**
Iron	123.7	0.738	**167.5**
Lead	0.562	0.018	**31.2**
Manganese	3.694	0.216	**17.10**
Nickel	0.457	0.067	**6.82**
Selenium	1.887	3.315	**0.57**
Vanadium	0.852	4.151	**0.21**
Zinc	72.10	1.487	**48.5**

The data from Table 5-20, combined with Figures 5-12 to 5-22 and the results of the statistical tests summarised in Table 5-19, provide information on the partitioning of heavy metals and selenium between egg yolk and albumen. Arsenic, selenium and vanadium were detected at significantly higher concentrations in the albumen than in the yolk ($p < 0.001$ for all), while cobalt, iron, lead, manganese, nickel and zinc concentrations were significantly higher in the yolk ($p < 0.001$ for all). However, it should be noted that cobalt and nickel were found almost entirely in the egg shell and concentrations in the egg yolk and albumen were comparatively low; therefore the results for partitioning of cobalt and nickel within egg contents should be treated with caution.

5.4 Discussion

5.4.1 Impacts of metal concentrations on egg parameters

Previous studies have shown that exposure of the laying bird to some metals can have a negative effect on the physical characteristics of the egg, with reports of reduced eggshell thickness associated with exposure to lead, vanadium and zinc, and decreased egg weight and size with excess concentrations of selenium and vanadium (see Section 5.1.2). No data regarding the effects of arsenic, cadmium, cobalt, copper, iron, manganese and nickel on the physical characteristics of the egg could be found. The present study suggests that increasing concentrations of cobalt, iron and nickel are associated with an increase in the wet weight of

albumen and a decrease in the ratio of yolk:albumen in the egg. No significant correlation was found between concentrations of these metals and wet weight of yolk, egg size, shell thickness or shell index. These results would suggest that cobalt, iron and nickel may have an impact on the intrinsic quality of the egg, as indicated by yolk:albumen ratio, at increased concentrations.

Although the concentrations of lead in the egg have been shown to be positively correlated with the wet weight of the albumen in this study (Section 5.3.1), lead concentration was not significantly correlated with yolk:albumen ratio, wet weight of yolk, egg size, shell thickness or shell index. The concentrations of lead measured in the eggs in this study do not therefore appear to be associated with any negative impacts on the physical characteristics of the egg. The majority of studies investigating the effects of lead on the egg report no reduction in shell thickness for a number of forms of lead (both organic and inorganic, at various concentrations (Haegele & Tucker, 1974; Haegele *et al.*, 1974; Pattee, 1984)), which is reflected in the results of this study. Dauwe *et al*. (2005) measured metal concentrations in the eggs of passerine species, as well as egg width, length, mass and volume, and eggshell thickness. There did not appear to be any decrease in eggshell thickness associated with lead even at the highest concentration of 3.21 μg/g dry weight in the egg, with shell thickness remaining relatively uniform in each of the eggs examined, although it should be noted that the study was conducted with passerine species and not seabirds. Total lead concentrations measured in the present study were all <1.6 μg/g dry weight. The concentrations of lead measured in the eggs in the present study are around half of those measured in passerine eggs and, as the concentrations measured in the passerine eggs did not negatively impact on the shell thickness, this may explain why an effect on eggshell thickness was not observed in this study - the lead concentrations seem unlikely to be of a high enough order to cause a reduction in eggshell thickness.

There were no significant relationships between the concentrations of the arsenic, cadmium, copper, manganese, vanadium and zinc in black-headed gull eggs measured in this study and the egg characteristics (wet weight of yolk, wet weight of albumen, yolk:albumen ratio, egg size, shell thickness or shell index). These results confirm the data found in the literature for arsenic and cadmium, which suggests that there is no significant relationship between these metals and the physical characteristics of the egg at the concentrations measured in this study. No literature data could be found regarding the effects of excess copper or manganese on the physical characteristics of the egg; the present study suggests that there is no significant relationship between these metals at the concentrations measured in this study and weight of components in the egg, egg size, shell thickness or shell index.

As an essential element, supplementation of the diet of laying birds with zinc has been shown to improve egg and eggshell quality (Moreng *et al.*, 1992; Sahin *et al.*, 2002). However, dietary exposure to excess zinc has been demonstrated to result in decreased egg shell thickness in one study, which reports a decrease in shell thickness of eggs laid by Japanese quail (*C. coturnix japonica*) after dosing of 15000 mg/kg body weight zinc for three days (Hussein *et al.*, 1988). Unfortunately, the study did not measure the amount of zinc that was sequestered to the eggs as a result of the dietary administration, and thus the amount of zinc measured in the egg in the present study cannot be compared directly with the dietary study. In another study, Dauwe *et al.* (2005) measured metal concentrations in the eggs of passerine species, as well as egg width, length, mass and volume, and eggshell thickness. The concentrations of zinc measured in egg contents in this study ranged from 31.5 µg/g to 286.1 µg/g dry weight and no decrease in eggshell thickness (or indeed any of the other egg parameters measured) was associated with these concentrations. These results would suggest that concentrations of up to 286 µg/g zinc dry weight do not have any significant impact on the eggshell thickness, although the study was conducted with passerine species and not seabirds. Total zinc concentrations measured in eggs in the present study were all <90 µg/g dry weight, and concentrations in egg contents all <75 µg/g. The comparatively low concentrations of zinc measured in the eggs in the present study may explain why an effect on eggshell thickness was not observed.

Transfer of selenium to eggs is considerable and dietary exposure to excess selenium has been associated with decreased egg weight in birds fed concentrations of selenium over 7 mg/kg body weight (Arnold *et al.*, 1973; Harr, 1978; Ort & Latshaw, 1978). In the studies reporting decreased egg weight after dietary exposure of the laying bird to selenium, no data for the amount of selenium sequestered into the eggs as a result of dietary exposure was reported. Unfortunately, this means that the amount of selenium measured in the egg in the present study cannot be directly equated to the amount that the birds are likely to have been exposed via the diet. The selenium concentrations measured in eggs in this study are considerably higher than those reported for seabird eggs in the literature, including those for other Laridae, and it would seem that the black-headed gulls examined in this study were exposed to relatively high levels of selenium (see Section 5.3.2). It should be noted, however, that a great deal of variation between wild bird species exists in terms of sensitivity to the teratogenic effects of selenium and, unfortunately, there is no data for the toxicity of selenium to the developing embryo specific to black-headed gulls. In addition, the majority of studies on the effects of selenium on breeding success of birds report embryo mortality and deformities as a result of exposure to excess selenium, rather than impacts on the physical characteristics of the egg. In the present

study, the hatching success and embryo and chick development was not examined and thus, although no relationship was found between selenium concentration in the egg and the physical egg characteristics, including egg weight, that is not to say that the selenium concentrations in the eggs examined here had no effect on the breeding success of the black-headed gulls. Future study would therefore be prudent, examining embryo development and hatching success in relation to selenium concentrations in eggs of black-headed gulls in these locations.

A reduction in egg production, decreased egg weight and decreased eggshell weight has been observed for domestic chickens (*Gallus* spp.) fed vanadium at a dose of 30-40 mg/kg feed (Ousterhout & Berg, 1981; Davis *et al.*, 1995; Bressman *et al.*, 2002). However, in another study with hens fed vanadium supplemented feed up to 100 mg/kg diet, egg weight and shell thickness were unaffected, even at the highest concentration. None of the studies measured the amount of vanadium that was sequestered to the eggs as a result of the dietary administration, and thus the amount of vanadium measured in the egg in the present study cannot be compared directly with the dietary studies. The vanadium concentrations measured in eggs in this study are over 150 times higher than those reported for gull eggs in the literature (see Section 5.4.3); however, these concentrations did not have a significant effect on egg weight, size or shell thickness. In the previous studies described, a significant reduction in feed consumption of the laying hen was observed during the experimental period, and it is possible that the effects on the egg may be due to this reduced feeding, rather than direct effects of vanadium. In the study in which egg weight and shell thickness were unaffected even at 100 mg/kg vanadium in the diet, no reduction in feed consumption was noted. Although the vanadium concentrations in eggs are relatively high in the present study, these concentrations are likely to have built up in the adult bird over time rather than the acute exposure to high vanadium doses in the diet as in the laboratory studies, and would be less likely to affect feeding. If the effects observed in some of the previous studies in the literature are attributable to reduction in feeding, this may explain why the concentrations measured in this study did not have an effect on the egg parameters.

5.4.2 Comparison of metal concentrations in eggs between sites

For the purposes of this section, metal concentrations in the eggs from the Poole and Raby sites will be compared with concentrations in the Lymington eggs pre-commercial harvesting ('Lymington Early' eggs), as these are more relevant for comparison with the Poole and Raby eggs, being from the first clutch laid and the colony as yet undisturbed by commercial

harvesting. The differences between pre- and post-collection eggs are dealt with in Section 5.4.4.

Differences between the Lymington and Poole sites

The results from this study show that black-headed gull eggs from the Poole colony have significantly higher average concentrations of arsenic, cobalt, copper, manganese, nickel, selenium and zinc than the eggs from the Lymington colony (Section 5.3.2), indicating that the black-headed gulls nesting at Poole are exposed to a higher levels of these metals than those nesting at Lymington. As this is not reflected by the point-source data (for which emissions of these metals are all higher at Lymington than Poole; Section 4.8.1, Table 4-13), it can be assumed that the majority of the heavy metal pollution around the Poole site comes from diffuse sources or historical pollution. The differences observed in the egg data in this study also reflect available literature data regarding sediment concentrations of metals in the Lymington and Poole areas (Bryan & Langston, 1992; Cundy & Croudace, 1995), with Poole sediment concentrations higher than Lymington sediment concentrations for copper (50 mg/kg in the Poole area compared with 24.5 mg/kg in the Lymington area), selenium (1.51 mg/kg compared with 0.41 mg/kg) and zinc (165 mg/kg compared with 93 mg/kg). Data for arsenic, cobalt and nickel concentrations in sediments of the Poole and Lymington areas are similar (14.1 mg/kg and 15.9 mg/kg, respectively for arsenic; 10 mg/kg and 11 mg/kg for cobalt; and 27 mg/kg for nickel at both sites). In contrast to the data for gull eggs in this study, concentrations of manganese reported in the literature are higher in sediments from the Lymington area than those from the Poole area (241 mg/kg and 185 mg/kg, respectively). However, it should be noted that the literature data for sediment concentrations are over 15 years old, and more up-to-date sediment concentrations of heavy metals would be beneficial to assessing the differences between metal contamination at the sites in this study.

In terms of runoff from urban areas and roads, the two sites are likely to be impacted to a similar degree; however, the Poole site is more likely to be impacted by agricultural runoff than the Lymington site (Section 4.8.2). Agricultural runoff has been demonstrated to contain elevated levels of arsenic, cobalt, copper, manganese, selenium and zinc from the application of fertilisers, pesticides and herbicides to arable land, and the administration of veterinary medicines and growth supplements, which are excreted in the waste products of livestock, along with nickel which is present in livestock faeces (see Section 4.2). The level of agricultural

runoff may therefore be a contributing factor to the fact that concentrations of these metals are all significantly higher in the Poole eggs than the Lymington eggs.

Both Lymington and Poole are important areas with regard to boating and shipping activities, both commercial and recreational (Sections 4.5.4 and 4.6.4). Based on the high level of shipping activity around the Lymington site (particularly in Southampton Water), there would be potential for the waters of the area to be more heavily polluted with metals such as copper and zinc as a result of the use of sacrificial zinc anodes and copper- and zinc-based antifoulant paints (Section 4.4). However, the fact that both copper and zinc concentrations in black-headed gull eggs in this study were higher in eggs from Poole than those from Lymington suggests that the gulls at the Poole site are exposed to higher levels of zinc and copper than those at the Lymington site. Poole Harbour has larger, more extensive marina facilities than Lymington Harbour, with moorings for a number of recreational craft and associated on-shore facilities for boat cleaning, painting, application of algicides, wood preservatives and varnishes, refuelling, engine repair and maintenance application. All of these marina-based activities have the potential to contribute significantly to metal pollution in estuary waters, which could explain why the waters in and around Poole may be more heavily contaminated with metals than those of Lymington and why the eggs of the black-headed gulls at Poole contained significantly higher mean concentrations of arsenic, cobalt, copper, manganese, nickel, selenium and zinc than the eggs of the black-headed gulls at Lymington. However, there are a large number of smaller marinas within the area of the Lymington site, which may contribute just as much collective pollution to the estuary waters as the fewer, more extensive facilities at Poole.

The historical pollution of Holes Bay in Poole Harbour with toxic metals discharges from industry in the 1970s and 80s is well documented (see Section 4.6.3), and metal concentrations in the waters and sediments of Holes Bay are still significantly higher than those in the rest of the Harbour, particularly for copper, lead, nickel and zinc (Boyden, 1975; Langston *et al.*, 2003). The enclosed and sheltered nature of Poole Harbour means that tidal exchange is restricted, leaving the Harbour poorly flushed and the dispersal of contaminants slow, making it particularly vulnerable to the effects of pollution (Langston *et al.*, 2003; Drake, 2007). In contrast, Lymington Estuary is hydraulically well-flushed. The combination of the heavy industrial activity carried out in Poole Harbour in the past, combined with the poor flushing of the estuary, is likely to have resulted in greater concentrations of metals (particularly copper, lead, nickel and zinc) in the sediment and waters of the area (see Section 4.6.3), and increased exposure of the black-headed gull nesting and feeding in the Poole area to metals in comparison to those nesting and feeding in the Lymington area.

Differences between the Raby site and the Lymington and Poole sites

Concentrations of copper, lead, manganese, nickel, selenium and zinc are significantly higher in black-headed gull eggs from the Raby colony than eggs from the Lymington colony (but not those from Poole; Section 5.3.2). The Raby site is very different to the Poole and Lymington sites in a number of ways (see Section 1.6), and the black-headed gulls nesting on the Raby Estate are exposed to very different types and degrees of metal pollution. There is little pollution in the area from current industrial and waste practices (Section 4.7.3) and emissions from point-sources are low (Table 4-13), neither is the site significantly impacted by diffuse pollution from road, urban or agricultural sources (Section 4.7.2). However, there are potential sources of metal pollution as a result of the geology of the area and its mining history.

The large granite body underlying the area may contribute copper, lead, nickel and zinc through natural weathering of rocks and soils (see Section 4.1). Owing to the granite base and mineral ores, the area of the Raby was mined extensively in the past for lead and, to a lesser degree, zinc. In fact, the black-headed gull colony examined in this study actually nest on old lead mining dams, near a reservoir. Selenium is also associated with waste and emissions from mining operations, being commonly produced as a by-product from mining and processing of many sulphide ores, such as those of lead, and manganese is a common contaminant in many mine waters (see Section 4.1) including those from lead mines in the North Pennines (Johnson & Younger, 2002). Copper, lead, nickel and zinc pollution in the Raby area may also be augmented by elevated concentrations in the soil and groundwater of the military firing range to the southwest of the site, as these metals are associated with bullets, pellets and incendiary devices (Section 4.7.2). The gulls are likely to feed from this area, and runoff could impact on adjacent areas and natural waters.

The geology of the area, its lead-mining past and the nearby military firing range, combined with the largely acidic soil conditions which allow for more rapid weathering and dissolution of metal-bearing minerals, means that the gulls nesting in the Raby area are likely to be exposed to elevated levels of copper, manganese, nickel, selenium, zinc and, most notably, lead. This supports the findings of this study, where all these metals were significantly higher in the eggs of gulls from the Raby colony in comparison to those from the gulls of the Lymington colony. The higher level of copper, lead, manganese and zinc pollution in the area is also reflected in literature data for metal concentrations in sediments of the River Tees, which runs through the area are feeds the reservoir which the colony nests alongside. Reported sediment concentrations in the Raby area are higher than those of sediments in the Lymington area for copper (120

mg/kg in the Raby area compared with 24.5 mg/kg in the Lymington area), lead (247 mg/kg compared with 45 mg/kg), manganese (396 mg/kg compared with 241 mg/kg) and zinc (472 mg/kg compared with 93 mg/kg; Davies *et al.*, 1991; Bryan & Langston, 1992; Cundy & Croudace, 1995). Conversely, nickel sediment concentrations reported for the two areas are similar (27 mg/kg for Raby and 26 mg/kg for Lymington). No data are available regarding the selenium concentration in sediments in the Raby area.

5.4.3 Potential impacts of metal concentrations in eggs on the breeding success of black-headed gulls

Comparisons are made with the concentrations of each metal measured in the eggs of black-headed gulls in this study and concentrations reported in the literature for eggs of other Laridae. The potential effects of the metal concentrations measured in this study on the breeding success of the black-headed gulls are assessed, utilising data from toxicity studies in the literature, reviewed in Section 5.1.2.

Arsenic

Mean concentrations of arsenic measured in the eggs of gulls in this study were higher for all sites than those reported for other gull species in the literature, with averages of 0.36-0.52 μg/g egg, compared to an average of 0.15 μg/g in the literature. The effects of arsenic on the breeding success of birds is dependant on the speciation as arsenic as arsenite (As^{3+}) is more toxic to embryos than arsenate (As^{5+}). Only one dietary study could be found regarding the effects of arsenic on breeding success, with no effect on egg parameters observed in eggs of domestic chickens fed up to 30 mg/kg body weight arsenite for 19 days (Holcman & Stibilj, 1997); no other indicators of breeding success were measured. The concentrations of arsenic measured in the egg as a result of this dietary dosing were 0.96 mg/kg dry weight in yolk and 0.3 mg/kg dry weight in albumen. Arsenic speciation was not examined in the present study; however, even assuming a worst-case scenario of all arsenic in the egg being present as arsenite, the concentrations of arsenic measured in black-headed gull eggs in this study are below those measured in the chicken eggs after the dietary administration. Thus, although the arsenic concentrations measured in eggs in this study are high relative to previous field studies with gull eggs, the concentrations measured are unlikely to have any negative effect on the egg. However, further study regarding the impacts of egg concentrations of arsenic on breeding

success, particularly with regard to effects on the developing embryo, is required in order to clarify the effects of arsenic on the breeding success of birds.

Cadmium

Concentrations of cadmium measured in black-headed gull eggs in this study were consistently lower than those reported in the literature for the eggs of other gull species, with averages of 0.009-0.012 μg/g egg dry weight in this study, compared to an average 0.026 μg/g in the literature. Other than suppression of egg production at very high concentrations of cadmium administered through the diet (over 200 mg/kg diet), there is no evidence in studies reported in the literature that cadmium has a negative effect on the breeding success of birds. The results from the present study reflect the low transfer of cadmium to eggs (Section 5.3.2), with concentrations below the limit of detection in many egg samples (<0.001 μg/g). The very limited transfer of cadmium to eggs and the subsequent low levels of cadmium measured in the black-headed eggs means that negative effects on the egg and embryo are highly unlikely.

Cobalt

Concentrations of cobalt measured in black-headed gull eggs in this study were slightly lower than those reported in the literature for the eggs of other gull species, with averages of 0.48-0.65 μg/g egg dry weight in this study, compared to an average 0.78 μg/g in the literature. Studies have demonstrated the transfer of cobalt from the laying bird to the egg (Agusa *et al.*, 2005; Dauwe *et al.*, 2005), but only one study could be found regarding the reproductive effects of cobalt in birds, reporting an LD_{50} of 38 μg/egg for embryos (Gilani & Alibhai, 1990) based on injection of cobalt direct into the egg. The effects of metals on reproductive success are less pronounced with dietary exposure than with direct injection into the egg (Section 5.1.2). Unfortunately, no data could be found in the literature reporting dietary or egg cobalt concentrations that have a negative impact on egg quality or embryo health and survival, and it is difficult to make an assessment as to the potential effects of the egg concentrations measured in black-headed gull eggs in the present study on breeding success. In this study, cobalt concentration in the egg was found to be significantly negatively correlated with yolk:albumen ratio (Section 5.3.1), indicating that increased concentration of cobalt may have a negative impact in the intrinsic quality of the egg. Further study regarding the impacts of egg concentrations of cobalt on egg production, egg quality and the developing embryo would be

prudent in order to make an accurate assessment of the effect of cobalt on the breeding success of birds.

Copper

Mean concentrations of copper measured in the eggs of gulls in this study are higher for all sites than those reported for other gull species in the literature, with averages of 7.9-11.6 μg/g egg dry weight in this study, compared to an average 4.7 μg/g in the literature. Very few studies have been carried out investigating the effects of copper on the reproductive success of birds. Copper injected into the yolk of domestic chicken (*Gallus* spp.) eggs has been demonstrated to have a negative impact on embryo development and lead to increased embryo mortality, at concentrations as low as 0.25 mg/kg egg for certain copper salts (Verrett, 1973; Verrett, 1974; Verrett, 1976). However, this study was based on direct administration of copper via egg injection, and dietary studies with copper concentrations of up to 480 mg/kg diet report no effect on egg production in the same species (Jackson, 1977). Unfortunately, the study did not measure the amount of copper sequestered to the eggs as a result of the dietary administration, and thus the amount of copper measured in the egg in the present study cannot be compared directly with the dietary studies. No dietary studies could be found in the literature reporting the effects of copper on egg parameters nor embryotoxicity and teratogenicity. It is therefore difficult to make inferences as to the potential of the copper concentrations measured in the present study to have a negative effect on reproductive success. Further study into the transfer of copper from the laying bird to the egg, and the impacts of egg concentrations of copper on egg quality and the developing embryo would be prudent.

Iron

In the present study, the concentrations of iron measured in eggs range from 117-199 μg/g egg dry weight. Unfortunately, no data for concentrations of iron in gull eggs, nor any other seabird species, from other field studies could be found in the literature. In other bird species, iron concentrations measured in eggs are variable with an average 85 μg/g in the eggs of birds of prey (Ambrose *et al.*, 2000) in a field study, and 26-47 μg/g egg in Japanese quail (*C. coturnix japonica*) in a laboratory study (Sanchez *et al.*, 1987). It would therefore seem that egg iron concentrations are variable between bird species and/or family. This phenomenon may occur as a result of diet, owing to the higher iron concentrations measured in eggs of birds of prey feeding on iron-rich meat than quail feeding mainly on seeds and berries, which are lower in

iron content. If diet is the main factor, this may explain why black-headed gull eggs have a high iron content, with the adult bird's iron-rich diet including shellfish and fish. Unfortunately, without any other data for iron concentrations in seabird eggs, nor any data regarding the sequestering of iron into eggs from the laying bird, it is difficult to put the concentrations measured in the present study into context. There are no studies in the literature examining the effects of elevated iron levels on the reproductive success of birds.

Lead

Mean concentrations of lead measured in the eggs of gulls in this study are higher for all sites than those reported for other gull species in the literature, with an average 0.23-0.52 μg/g egg in this study for Lymington and Poole eggs and 1.52 μg/g egg for Raby eggs, compared to an average of 0.25 μg/g in the literature. The toxicity of lead to adult birds depends on the form of lead and the species, age and sex of the bird. Based on dietary studies, lead can suppress egg production from concentrations as low as 1 mg/kg diet, and may cause reduced egg quality and a decrease in hatching rate at dietary concentrations of over 100 mg/kg. Unfortunately, none of the dietary studies measured the amount of lead sequestered to the eggs as a result of the dietary administration, and thus the amount of lead measured in the egg in the present study cannot be compared directly with the dietary studies. It is therefore difficult to make inferences as to the potential of the lead concentrations measured in the present study to have a negative effect on reproductive success. With the vast majority of studies focusing on the toxicity of lead to adult birds, further investigation into the impacts of egg concentrations of lead on the egg and the developing embryo is required to assess the effects on the breeding success of birds.

Manganese

Mean concentrations of manganese in the eggs of gulls in this study are of a similar order to those reported for other gull species in the literature, with an average 3.36-5.19 μg/g egg in this study, compared to an average of 4.38 μg/g in the literature. Exposure to excess manganese has been demonstrated to cause teratogenic effects such as abnormally small, poorly developed and deformed embryos. An LD_{50} of 765 μg/egg has been determined (Gilani & Alibhai, 1990), however this is based on injection of manganese direct into the egg, and there is no data in the literature reporting dietary or egg manganese concentrations that have a negative impact on egg quality or embryo health and survival. Based on the limited data available, the concentrations of

manganese measured in the black-headed gull eggs in this study do not seem likely to be of a level to have an adverse effect on breeding success.

Nickel

Nickel concentrations in the eggs of gulls in this study average between 8.1 and 11.9 μg/g egg. Unfortunately, no data for dry weight concentrations of nickel in gull eggs from other field studies could be found in the literature. However, concentrations in the eggs of other seabird species have been reported, with a mean concentration of 1.14 μg/g dry weight reported in eggshells. The concentrations of nickel measured in eggshells in this study were all higher than this literature value. Data regarding the effects of nickel on breeding birds was found in only one study, which reports an LD_{50} of 3.6 μg/g embryo for nickel injected into chick embryos (Ridgway & Karnofsky, 1952). However, the effects of metals on reproductive success are usually less pronounced with dietary exposure than direct injection into the egg or embryo, and no data could be found in the literature reporting dietary or egg nickel concentrations that have a negative impact on egg quality or embryo health and survival. In toxicity studies with newly hatched and adult birds, young birds appear to be more sensitive than adults, with dietary doses in excess of 800 mg/kg diet resulting in significant mortality of young birds, but having no effect on the health or reproductive success of adult birds. Based on this limited data, the concentrations of nickel measured in the black-headed gull eggs in this study do not seem to be of a level to have an adverse effect on breeding success. However, further study regarding the impacts of egg concentrations of nickel on egg production, egg quality and the developing embryo is required to make an accurate assessment of the effect of nickel on the breeding success of birds.

Selenium

Mean concentrations of selenium measured in the eggs of gulls in this study were higher for all sites than those reported for other gull species in the literature, with an average 5.5-8.1 μg/g egg dry weight in this study, compared to an average of 2.4 μg/g dry weight in the literature. Reproductive success is more sensitive to selenium toxicity than survival of young and adult birds and, in general, embryo deformities and hatching failure occur when the selenium concentration in the egg exceeds 3 μg/g wet weight (Section 5.1.2). The metal concentrations in the present study are calculated on a dry weight basis and, as metals will be more concentrated in dry weight samples, the threshold for dry weight egg concentrations will be higher than the

wet weight value. In the present study, the average water content of the black-headed gull eggs was 75%; thus the metal concentrations will be around four times higher in the dry content than the wet content, and an approximate threshold for selenium concentrations in eggs on a dry weight basis can therefore be taken as approximately 12 μg/g. The concentrations of selenium in black-headed gull eggs measured in the present study, although consistently higher than those reported in the literature for other seabird species, are therefore unlikely to be of a sufficient level to pose a threat to the reproductive success of the birds.

Vanadium

Concentrations of vanadium measured in this study were over 150 times higher than those reported in the literature for other gull species, with an average 64-86 μg/g egg in this study, compared to an average of 0.052 μg/g egg in the literature; however, this literature value is based on only one study, as only one previous field study has reported vanadium concentrations in gull eggs. Other literature data for vanadium the eggs of other seabird species are also over 80 times lower, even for the highest concentration recorded. The only data regarding the effects of excess vanadium on reproductive success are for commercial laying chickens (*Gallus* spp.), with reduced egg production, decreased egg weight, decreased eggshell weight and reduced hatchability of eggs observed for hens fed vanadium at a dose of 30-40 mg/kg diet (Kubena *et al.*, 1980; Ousterhout & Berg, 1981; Davis *et al.*, 1995; Bressman *et al.*, 2002). An increase in embryo mortality has also been reported as a result of laying hens fed vanadium-supplemented feed at a dose of 60 mg/kg diet (Bressman *et al.*, 2002). Unfortunately, the study did not measure the amount of vanadium that was sequestered to the eggs as a result of the dietary administration, and thus the amount of vanadium measured in the egg in the present study cannot be compared directly with the dietary study. It is therefore difficult to assess the potential effects of the level of vanadium measured in the gull eggs in this study on reproductive success. As the vanadium concentrations in the eggs examined in this study were of a similar order, irrespective of the site sampled and the associated potential sources of pollution, the results would suggest that the concentrations of vanadium measured in the eggs in this study may be considered to be 'normal' for black-headed gull eggs. In addition, the vanadium concentrations measured in eggs in this study did not have a negative effect on any of the physical characteristics of the egg (see Section 5.4.1). Shellfish are a major source of vanadium in the diet of seabirds; however, most seabirds have a diet consisting of a proportion of shellfish, and there is nothing to suggest why the black-headed gull should be exposed to an increased amount of vanadium through the amount of shellfish consumed compared to the other seabirds

looked at in the literature. However, black-headed gulls spend much more time on land than other seabird species, and it may be the case that these birds are exposed to vanadium as a result of the amount of time they spend foraging on land. Major sources of vanadium in the environment are burning of fossil fuels and the use in mineral fertilisers applied to agricultural land (see Section 4.2). It may be the case that black-headed gulls feeding on land as well as at sea are exposed to more anthropogenic sources of vanadium than other seabirds, as they are frequently found foraging in urban areas and on farmland (Section 1.5). Further investigation into the concentration of vanadium in black-headed gulls from a variety of different locations would be prudent, as would study into the transfer of vanadium from the laying bird to the egg, and the effects of egg concentrations of vanadium of the breeding success of seabirds.

Zinc

Zinc concentrations measured in the eggs of gulls in this study are similar or slightly higher than those reported for other gull species in the literature, with an average 64.3-85.5 μg/g egg in this study, compared to an average of 65.5 μg/g in the literature. Exposure to excess zinc has been shown to decrease egg production in birds, and zinc is often added to the diet by commercial poultry managers to reduce egg deposition and improve long-term egg production. When injected into the yolk of domestic chicken (*Gallus* spp.) eggs, zinc has been demonstrated to impair embryo development and lead to increased embryo mortality, with an LD_{50} of 1.0 μg/g egg (Birge & Roberts, 1976). However, the effects of metals on reproductive success are usually less pronounced with dietary exposure than direct injection into the egg or embryo (Section 5.1.2). In dietary studies, concentrations of 10000 mg/kg feed and above have been shown to significantly decrease or completely halt laying, with the eggs produced having lower quality shells and decreased hatchability (Palafox & Ho-A, 1980; Decuypere *et al.*, 1988; Hussein *et al.*, 1988). None of the studies measured the amount of zinc sequestered to the eggs as a result of the dietary administration, and thus the amount of zinc measured in the egg in the present study cannot be compared directly with the dietary studies. Based on the limited data in the literature, combined with the fact that the zinc concentrations measured in eggs in this study are of a similar order to those measured in previous field studies, it seems unlikely that the concentrations of zinc measured in the black-headed gull eggs in this study are of a level that would have an adverse effect on breeding success. However, further study into the impacts of egg concentrations of zinc on egg quality and the developing embryo would be prudent.

5.4.4 Differences in metal concentrations in black-headed gull eggs pre- and post-harvesting

Comparison of eggs from the Lymington site collected before the commercial harvesting period ('Lymington Early') with those collected after the harvesting period ('Lymington Late'; i.e. those eggs that would be allowed to develop and hatch) was made to assess whether heavy metals and selenium become concentrated in the egg as the female continues to lay. Concentrations of organic pollutants have been shown to increase through the laying sequence, with higher concentrations reported in the last-laid egg compared with the first-laid (Becker, 1989); however, few studies have examined the effects of laying sequence on the concentrations of heavy metals in the eggs. The two studies that have examined the impact of laying order on metal concentrations in the egg have reported no clear relationship between laying order and egg concentrations of any of the metals examined in the present study (Becker *et al.*, 1989; Dauwe *et al.*, 2005). The effect of commercial egg harvesting and subsequent replacement laying on the concentrations of heavy metals and selenium in eggs has not been previously examined.

The results of this study reveal a significantly higher mean concentration in the post-collection 'Lymington Late' eggs than the pre-collection 'Lymington Early' eggs for cobalt, iron and nickel. Concentrations of arsenic, lead, manganese and selenium were also higher in post-collection eggs than pre-collection eggs from Lymington, although the differences were not statistically significant. There were no metals for which the concentrations in the pre-collection eggs were significantly higher than the post-collection eggs. The results suggest that cobalt, iron and nickel concentrations increase with laying, when replacement laying is necessary as a result of commercial egg harvesting. As suggested by previous authors regarding increase of organic pollutants through the laying sequence, it is likely that the increase in these metals with laying occurs because birds produce their first-laid egg or eggs mainly from recent dietary uptake, whereas body reserves contribute more to later eggs (Mineau, 1982). In the case of the black-headed gulls nesting at Lymington, the female birds will lose a substantial portion of their body burden of pollutants through repeated laying, which requires mobilising body reserves and increased feeding, both of which may lead to increased concentration of metals in the laying bird, which will subsequently be sequestered into eggs. In addition, continued relaying may require the laying female to mobilise body reserves and contaminants that have been obtained prior to breeding, and may therefore have been acquired from an entirely different area in which the bird may have been exposed to different types and degrees of pollution to those around the breeding site (see Chapter 4).

Previous studies have not found an increase in heavy metal concentration with laying order. However, these studies examined only a limited number of eggs: in the case of the study with seabirds (Becker, 1989) an average clutch of three eggs was examined, and in the case of the passerine study (Dauwe *et al.*, 2005), the first eggs of each clutch were examined. As previously mentioned, the present study is the first to examine the phenomenon of changing concentrations of metals with repeated replacement laying, as is induced by commercial harvesting of eggs, and the limited number of eggs examined in previous studies may explain why they did not find a significant increase in the concentrations of these metals with laying order.

The post-collection Lymington eggs have been demonstrated to have significantly higher concentrations of cobalt, iron and nickel compared to pre-collection eggs, and the concentrations of these metals in the egg have been shown to be significantly negatively correlated with yolk:albumen ratio (Section 5.3.1). The concentrations of the other metals examined in this study have been shown to have no significant effect on the physical characteristics of the egg, and are not of a level thought to have a negative impact on breeding success (Section 5.4.3). However, the concentration of these metals in eggs as a result of commercial egg harvesting could have profound consequences for black-headed gulls nesting in areas with higher levels of exposure to pollution, or following a pollution incident, as the concentration of metals in the egg could then result in egg concentrations sufficient to have a negative impact on the egg or the developing embryo.

5.4.5 Metal partitioning in the egg

Eggshell:egg contents

As previously discussed (Section 5.1.3, Table 5-12), the eggshell:egg ratios of heavy metals in bird eggs have been reported by a number of authors. Table 5-21 provides ratios generated by other authors for different species of gull, along with data for the black-headed gull in this study.

Table 5-21 Eggshell:egg ratios of metals in gull species, including data from this study

Metal	Herring gull (a)	Black-tailed gull (b)	Audouin's gull (c)	Black-headed gull (this study)
Arsenic	-	-	-	0.16
Cadmium	5.0	0.62	-	2.5
Cobalt	-	17.2	-	15.4
Copper	-	0.13	0.83	1.3
Iron	-	-	-	0.80
Lead	0.1	2.65	-	0.88
Manganese	1.5	0.62	0.17	0.28
Nickel	-	-	-	33.2
Selenium	0.13	0.13	-	0.35
Vanadium	-	0.27	-	0.91
Zinc	-	0.01	0.11	0.02

(a) Burger, 1994 (b) Agusa *et al*., 2005 (c) Morera *et al*., 1997

The data in Table 5-21 allow for comparison of the eggshell:egg ratios found in this study with the ratios of metals in eggs of other gull species reported by previous authors. Cadmium ratios reported by previous authors are variable, with higher concentrations reported in the eggshell than the contents for the herring gull (*L. argentatus*; Burger, 1994), and higher concentrations reported in the egg contents than the shell for the black-tailed gull (*L. crassirostris*; Agusa *et al.*, 2005). In this study, the eggshell:egg ratio of 2.5 and the results of the statistical tests show that, for the black-headed gull, cadmium concentrations were significantly higher in the egg shell than egg contents. However, the concentrations of cadmium were low in general, and the data should be treated with some caution as the concentration of cadmium was below the limit of detection (<0.001 μg/g) in many of the samples, with just one or two measurable concentrations biasing the mean concentration (as reflected in the standard errors).

Concentrations of cobalt were found to be higher in eggshell than egg contents for the black-tailed gull (*L. crassirostris*; Agusa *et al.*, 2005), as was also found for the black-headed gull in this study (eggshell:egg ratio 15.4). For copper, the data from this study reports an almost 1:1 ratio and no significant difference between copper concentrations in the egg shell and contents. Conversely, the ratios reported for other gull species reflect lower concentrations in the shell than the contents (ratios of 0.13 and 0.83). Manganese eggshell:egg ratios from previous studies with gull species differ, with higher concentrations of manganese found in the egg shell compared to the egg contents for the herring gull (*L. argentatus*; Burger, 1994), but higher concentrations in the egg contents compared to the shell for the black-tailed gull (*L.*

crassirostris; Agusa *et al.*, 2005) and Audouin's gull (L. audouinii; Morera *et al.*, 1997). For the black-headed gull in this study, manganese was present more in the egg contents than in the eggshell overall (eggshell:egg ratio = 0.28), with concentrations in yolk significantly higher than those in both albumen and shell. However, concentrations in albumen were less than those in eggshell, indicating that the most significant part of the egg in terms of manganese partitioning is the yolk (see also 'Yolk:albumen ratio', below) .

Eggshell:egg ratios for zinc in this study are similar to those ratios reported for gull eggs in other studies, with zinc partitioned mainly in the egg contents (ratio 0.02), with the highest concentrations of zinc found in the egg yolk. Selenium is reported to be detected mainly in the egg contents in previous studies with gulls, which is reflected in this study, with black-headed gull eggs exhibiting a eggshell:egg ratio of 0.35 for selenium and a significantly higher mean concentration of selenium in egg contents than eggshell.

Results from previous studies with gulls regarding the partitioning of lead are conflicting, with lead concentrations reported to be higher in the shell for the black-tailed gull (*L. crassirostris*; Agusa *et al.*, 2005), and higher in the contents for the herring gull (*L. argentatus*; Burger, 1994). In the present study, lead concentrations were higher in the egg contents overall (eggshell:egg ratio 0.88), although specifically concentrations were significantly higher in the yolk than either the albumen or the shell, and concentrations in the shell were actually higher than those in the albumen. Thus the results from this study with black-headed gulls show that the yolk is the most important part of the egg with respect to lead concentration.

Vanadium is partitioned mainly in the egg contents in the black-tailed gull (*L. crassirostris*; Agusa *et al.*, 2005). In this study, concentrations of vanadium were found to be slightly higher in the egg contents compared to the shell (eggshell:egg ratio 0.91), and were significantly higher in albumen than any other part of the egg, although shell concentrations were higher than those in yolk. Thus, the albumen would seem to be the most important part of the egg with respect to vanadium partitioning (see 'Yolk:albumen ratio', below).

Unfortunately no previous data could be found for eggshell:egg ratios of arsenic, iron or nickel in gull eggs. In this study, arsenic and iron were detected mainly in the egg contents (eggshell:egg ratios 0.16 and 0.80, respectively, with iron being found mainly in the yolk), and nickel was detected mainly in the eggshell (eggshell:egg ratio 33.2).

Yolk:albumen ratio

Although the eggshell:egg ratios of heavy metals in bird eggs have been reported by a number of authors, only one study could be found reporting partitioning of metals between egg yolk and albumen: Magat and Sell (1979) found that selenium binds preferentially to albumen, rather than yolk. No further studies with metals could be found in the literature where the authors separated the egg contents to allow for separate analysis of yolk and albumen. Separation of the two egg components allows for the examination of the partitioning of heavy metals within the egg contents, as opposed to only between the shell and the contents as a whole.

This study has shown that, within the egg, arsenic, selenium and vanadium are partitioned mainly in the albumen, and cobalt, iron, lead, manganese, nickel and zinc partitioned mainly in the yolk. However, it should be noted that, as cobalt and nickel were found almost entirely in the egg shell, the concentrations of these metals in the egg contents were very low and the results for their partitioning within the egg contents should be treated with caution. The results for selenium partitioning in egg contents in the present study reflect those reported by previous authors (Magat & Sell, 1979).

5.5 Summary

This study has shown black-headed gull eggs to provide a good indication of local sources of pollution for a number of metals, with concentrations of lead and zinc particularly high in eggs from the Raby site, where mining for lead and zinc has been carried out in the past, and concentrations of most metals higher in eggs from the Poole site, most likely owing to diffuse pollution from local agricultural land, historical pollution of Poole Bay and the enclosed, sheltered nature of the harbour, which limits the dispersion of contaminants.

The concentrations of arsenic, copper, lead, selenium and, most notably, vanadium, measured in black-headed gull eggs in this study are consistently high compared to concentrations measured in eggs of other gull species in previous studies. In spite of the relatively high concentrations of these metals, there are no significant correlations between the concentrations of these metals and the quality of the eggs, as indicated by egg size, yolk:albumen ratio, shell thickness and shell index. Data regarding the effects of many heavy metals on the breeding success of birds are limited, and the review of the literature has highlighted the need for more information regarding the sequestering of heavy metals into eggs and the impacts on breeding success.

Increasing concentrations of cobalt, iron and nickel in the egg are significantly correlated with a decrease in the intrinsic quality of black-headed gull eggs, as indicated by yolk:albumen ratio, and both the concentrations of these metals and the yolk:albumen ratio are impacted by commercial egg collection, with eggs from the uncollected site and pre-collection eggs from the collected site containing significantly lower concentrations of cobalt, iron and nickel and significantly higher ratio of yolk:albumen than post-collection eggs from the collected site. The relative amount of yolk in the egg is considered to provide a good indication of the quality of the egg, as the yolk is the food reserve for the developing embryo. In addition to providing the majority of the necessary nutrients for the developing embryo, large-yolked eggs provided the newly hatched chick with more residual yolk reserves, which is crucial for survival during the first few days of life (Parsons, 1970; Lundberg & Väisänen, 1979). The results of this study therefore suggest that the commercial collection of black-headed gull eggs is having a negative impact on the potential breeding success of the gulls, as eggs are of a lower quality and likely to produce smaller hatchlings with less yolk reserves, and the concentrations of the potentially toxic heavy metals cobalt, iron and nickel are also increased as a result of repeated relaying.

Information has been provided regarding the partitioning of heavy metals and selenium, both between egg shell and contents and within the egg contents themselves. The difference in concentrations of heavy metals and selenium between different parts of the egg has highlighted the importance of analysing all components of the egg in order to provide a true indication of the total concentration of metals in eggs.

CHAPTER 6. HEAVY METALS AND SELENIUM IN BLACK-HEADED GULL FEATHERS

6.1 Introduction

General information regarding the environmental fate and toxicity to birds of the metals covered in this study is dealt with in Section 5.1.1.

The proportion of the body burden that is in feathers is relatively constant for any metal (Burger, 1993) and for some metals, for example mercury, concentrations in feathers can be higher, and hence easier to detect and quantify, than the metals present in blood or other tissue samples (Cahill *et al.*, 1998; Dauwe *et al.*, 2002a). Although a number of experiments have been undertaken to investigate the potential use of feathers as a method of assessing the internal tissue concentrations of mercury in birds, relatively few published data are available for other metals. In particular, there is a need to discriminate between the quantities of metal in feathers from the diet and from atmospheric deposition. It has been demonstrated experimentally that mercury in feathers is strongly bonded and concentrations are not affected by storage or vigorous treatments (Appelquist *et al.*, 1984), and Weyers *et al*. (1988) demonstrated with electromicroscopic photos that, for lead and cadmium, various small metal particles remained on the surface of feathers even after vigorous washing with propanon or Triton-X-100 in an ultrasonic bath. However, the case may be different for other metals that enter feathers from the blood stream during feather growth, and further research is needed to assess the potential of feathers as tools for monitoring internal metal contamination.

Studies have been carried out to investigate the link between heavy metal concentrations in feathers and the concentrations in internal tissues (Goede & de Bruin, 1986; Burger, 1993; Lewis *et al.*, 1993); however results have been inconclusive and, in some cases, conflicting. For metals other than mercury, lead and cadmium, feather concentrations are poorly documented and further study is required to assess the suitability of feathers as biomonitors of these metals.

Tables 6-1 to 6-11 below provide a summary of concentrations of heavy metals and selenium measured in seabird feathers in previous field studies reported in the literature. Unfortunately, no data are available for cobalt concentrations in seabird feathers. Data provided are for dry weight concentrations from studies worldwide, undertaken from 1990 onwards.

Table 6-1 Summary of literature data for arsenic concentrations in seabird feathers

Species	Location	Mean concentration ppm (dry weight)	Study year	Notes	Reference
Franklin's gull ***Larus pipixcan***	Minnesota, USA	0.103	1994	Adult	Burger and Gochfeld, 1999
	Minnesota, USA	0.0546	1994	Juvenile	Burger and Gochfeld, 1999
Sooty tern ***Sterna fuscata***	Midway Atoll, Pacific	0.124	1997	Adult	Burger and Gochfeld, 2000a
Grey-backed tern ***Sterna lunata***	Midway Atoll, Pacific	0.146	1997	Adult	Burger and Gochfeld, 2000a
White tern ***Gygis alba***	Midway Atoll, Pacific	0.459	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	0.668	1997	Juvenile	Burger and Gochfeld, 2000a
Brown noddy ***Anous stolidus***	Midway Atoll, Pacific	0.332	1997	Adult	Burger and Gochfeld, 2000a
Bonin petrel ***Pterodroma hypoleuca***	Midway Atoll, Pacific	0.0595	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	0.107	1997	Juvenile	Burger and Gochfeld, 2000a
Christmas shearwater ***Puffinus nativitatis***	Midway Atoll, Pacific	0.36	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	1.56	1997	Juvenile	Burger and Gochfeld, 2000a
Wedge-tailed shearwater ***Puffinus pacificus***	Midway Atoll, Pacific	0.0881	1997	Adult	Burger and Gochfeld, 2000a
Laysan albatross ***Diomedea immutabilis***	Midway Atoll, Pacific	0.11	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	0.182	1997	Juvenile	Burger and Gochfeld, 2000a
Black-footed albatross ***Diomedea nigripes***	Midway Atoll, Pacific	0.208	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	1.014	1997	Juvenile	Burger and Gochfeld, 2000a
Red-footed booby ***Sula sula***	Midway Atoll, Pacific	0.125	1997	Adult	Burger and Gochfeld, 2000a
Red-tailed tropicbird ***Phaethon rubricauda***	Midway Atoll, Pacific	0.0567	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	0.198	1997	Juvenile	Burger and Gochfeld, 2000a
Great frigatebird ***Fregata minor***	Midway Atoll, Pacific	0.158	1997	Adult	Burger and Gochfeld, 2000a
Osprey ***Pandion haliaetus***	Florida, USA	0.136	200-2001	Adult	Lounsbury-Billie *et al*., 2008
Gentoo penguin ***Pygoscelis papua***	Antarctica	0.88	2002	Adult	Metcheva *et al*., 2006
Chinstrap penguin ***Pygoscelis antarctica***	Antarctica	0.45	2002	Adult	Metcheva *et al*., 2006

Table 6-2 Summary of literature data for cadmium concentrations in seabird feathers

Species	Location	Mean concentration ppm (dry weight)	Study year	Notes	Reference
Herring gull ***Larus argenatatus***	Northeast Siberia	0.57	1993	Adult	Kim *et al*., 1996
Franklin's gull ***Larus pipixcan***	Interior North America	0.568	1994	Adult	Burger, 1996
	Interior North America	0.413	1994	Juvenile	Burger, 1996
	Minnesota, USA	0.409	1994	Adult	Burger and Gochfeld, 1999
	Minnesota, USA	0.117	1994	Juvenile	Burger and Gochfeld, 1999
Laughing gull ***Larus atricilla***	New York, USA	0.197	1992	Adult	Gochfeld *et al*., 1996
Black-tailed gull ***Larus crassirostris***	Japan	0.044	1999-2001	Adult	Agusa *et al*., 2005
Parasitic jaeger ***Stercorarius parasiticus***	Northeast Siberia	0.48	1993	Adult	Kim *et al*., 1996
Common tern ***Sterna hirundo***	New York, USA	0.2	1990	Adult	Gochfeld *et al*., 1991
	New York, USA	0.12	1991	Adult	Burger *et al*., 1992a
	Massachusesetts, USA	0.42	1991	Adult	Burger *et al*., 1992a
	New York, USA	0.11	1991	Juvenile	Burger and Gochfeld, 1992a
Sooty tern ***Sterna fuscata***	Puerto Rico	0.22	1990	Adult	Gochfeld *et al*., 1991
	Hawaii	0.147	1990	Adult	Burger *et al*., 1992c
	Johnston Atoll, Pacific	0.131	1990	Adult	Burger *et al*., 1992c
	Midway Atoll, Pacific	0.0734	1997	Adult	Burger and Gochfeld, 2000a
Roseate tern ***Sterna dougallii***	New York, USA	0.16	1991	Adult	Burger *et al*., 1992a
Grey-backed tern ***Sterna lunata***	Midway Atoll, Pacific	0.095	1997	Adult	Burger and Gochfeld, 2000a
White tern ***Gygis alba***	Midway Atoll, Pacific	0.216	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	0.382	1997	Juvenile	Burger and Gochfeld, 2000a
Black skimmer ***Rynchops niger***	New York, USA	0.11	1990	Adult	Gochfeld *et al*., 1991
	New York, USA	0.06	1991	Adult	Burger and Gochfeld, 1992b
Brown noddy ***Anous stolidus***	Hawaii	0.2	1990	Adult	Burger, 1993b
	Hawaii	0.08	1990	Juvenile	Burger, 1993b
	Midway Atoll, Pacific	0.274	1997	Adult	Burger and Gochfeld, 2000a
Bonin petrel ***Pterodroma hypoleuca***	Midway Atoll, Pacific	0.129	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	0.103	1997	Juvenile	Burger and Gochfeld, 2000a
Christmas shearwater ***Puffinus nativitatis***	Midway Atoll, Pacific	0.95	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	0.404	1997	Juvenile	Burger and Gochfeld, 2000a
Wedge-tailed shearwater ***Puffinus pacificus***	Hawaii	0.29	1990	Adult	Burger *et al*., 1992c
	Johnston Atoll, Pacific	0.32	1990	Adult	Burger *et al*., 1992c
	Midway Atoll, Pacific	0.0709	1997	Adult	Burger and Gochfeld, 2000a
Laysan albatross ***Diomedea immutabilis***	Midway Atoll, Pacific	0.364	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	0.496	1997	Juvenile	Burger and Gochfeld, 2000a
Black-footed albatross ***Diomedea nigripes***	Midway Atoll, Pacific	0.152	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	1.58	1997	Juvenile	Burger and Gochfeld, 2000a

Table 6-2 cont.

Species	Location	Mean concentration ppm (dry weight)	Study year	Notes	Reference
Red-footed booby ***Sula sula***	Johnston Atoll, Pacific	0.13	1990	Adult	Burger and Gochfeld, 1991b
	Johnston Atoll, Pacific	0.14	1990	Adult	Burger and Gochfeld, 1991b
	Midway Atoll, Pacific	0.0513	1997	Adult	Burger and Gochfeld, 2000a
Red-tailed tropicbird ***Phaethon rubricauda***	Midway Atoll, Pacific	0.0552	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	0.115	1997	Juvenile	Burger and Gochfeld, 2000a
Great frigatebird ***Fregata minor***	Midway Atoll, Pacific	0.204	1997	Adult	Burger and Gochfeld, 2000a
Arctic loon ***Gavia arctica***	Northeast Siberia	0.42	1993	Adult	Kim *et al*., 1996
Gentoo penguin ***Pygoscelis papua***	Antarctica	0.21	2002	Adult	Metcheva *et al*., 2006
Chinstrap penguin ***Pygoscelis antarctica***	Antarctica	0.3	2002	Adult	Metcheva *et al*., 2006

Table 6-3 Summary of literature data for cobalt concentrations in seabird feathers

Species	Location	Mean concentration ppm (dry weight)	Study year	Notes	Reference
Osprey ***Pandion haliaetus***	Florida, USA	0.094	200-2001	Adult	Lounsbury-Billie *et al*., 2008

Table 6-4 Summary of literature data for copper concentrations in seabird feathers

Species	Location	Mean concentration ppm (dry weight)	Study year	Notes	Reference
Herring gull ***Larus argenatatus***	Northeast Siberia	12.2	1993	Adult	Kim *et al*., 1996
Black-tailed Gull ***Larus crassirostris***	Japan	5.6	1999-2001	Adult	Agusa *et al*., 2005
Glaucous gull ***Larus hyperboreus***	Northeast Siberia	12	1993	Adult	Kim *et al*., 1996
Sabine's gull ***Xema sabin***	Northeast Siberia	17.1	1993	Adult	Kim *et al*., 1996
Parasitic jaeger ***Stercorarius parasiticus***	Northeast Siberia	13.9	1993	Adult	Kim *et al*., 1996
Long-tailed jaeger ***Stercorarius longicaudus***	Northeast Siberia	18.1	1993	Adult	Kim *et al*., 1996
Arctic tern ***Sterna paradisaea***	Northeast Siberia	17.5	1993	Adult	Kim *et al*., 1996
Black skimmer ***Rynchops niger***	New York, USA	27.4	1991	Adult	Burger and Gochfeld, 1992b
Arctic loon ***Gavia arctica***	Northeast Siberia	41.5	1993	Adult	Kim *et al*., 1996
Osprey ***Pandion haliaetus***	Florida, USA	8.05	200-2001	Adult	Lounsbury-Billie *et al*., 2008
Gentoo penguin ***Pygoscelis papua***	Antarctica	17	2002	Adult	Metcheva *et al*., 2006
	Antarctica	16	2003	Adult	Metcheva *et al*., 2006
Chinstrap penguin ***Pygoscelis antarctica***	Antarctica	19	2002	Adult	Metcheva *et al*., 2006
	Antarctica	18	2003	Adult	Metcheva *et al*., 2006

Table 6-5 Summary of literature data for iron concentrations in seabird feathers

Species	Location	Mean concentration ppm (dry weight)	Study year	Notes	Reference
Herring gull ***Larus argenatatus***	Northeast Siberia	18.6	1993	Adult	Kim *et al*., 1996
Glaucous gull ***Larus hyperboreus***	Northeast Siberia	13.4	1993	Adult	Kim *et al*., 1996
Sabine's gull ***Xema sabin***	Northeast Siberia	81.8	1993	Adult	Kim *et al*., 1996
Parasitic jaeger ***Stercorarius parasiticus***	Northeast Siberia	17.8	1993	Adult	Kim *et al*., 1996
Long-tailed jaeger ***Stercorarius longicaudus***	Northeast Siberia	35.7	1993	Adult	Kim *et al*., 1996
Arctic tern ***Sterna paradisaea***	Northeast Siberia	35.6	1993	Adult	Kim *et al*., 1996
Arctic loon ***Gavia arctica***	Northeast Siberia	53	1993	Adult	Kim *et al*., 1996
Osprey ***Pandion haliaetus***	California, USA	424	1992-1996	Adult	Cahill *et al*., 1998
Gentoo penguin ***Pygoscelis papua***	Antarctica	56	2002	Adult	Metcheva *et al*., 2006
	Antarctica	46	2003	Adult	Metcheva *et al*., 2006
Chinstrap penguin ***Pygoscelis antarctica***	Antarctica	53	2002	Adult	Metcheva *et al*., 2006
	Antarctica	42	2003	Adult	Metcheva *et al*., 2006

Table 6-6 Summary of literature data for lead concentrations in seabird feathers

Species	Location	Mean concentration ppm (dry weight)	Study year	Notes	Reference
Herring gull ***Larus argentatus***	New Jersey, USA	0.81	1991	Juvenile	Burger *et al*., 1992d
Franklin's gull ***Larus pipixcan***	Interior North America	2.868	1994	Adult	Burger, 1996
	Interior North America	0.961	1994	Juvenile	Burger, 1996
	Minnesota, USA	2.67	1994	Adult	Burger and Gochfeld, 1999
	Minnesota, USA	0.427	1994	Juvenile	Burger and Gochfeld, 1999
Laughing gull ***Larus atricilla***	New York, USA	2.824	1992	Adult male	Gochfeld *et al*., 1996
	New York, USA	2.377	1992	Adult female	Gochfeld *et al*., 1996
Black-tailed gull ***Larus crassirostris***	Japan	0.754	1999-2001	Adult	Agusa *et al*., 2005
Common tern ***Sterna hirundo***	New York, USA	1.62	1990	Adult	Gochfeld *et al*., 1991
	New York, USA	3.6	1991	Adult	Burger *et al*., 1992a
	Massachusesetts, USA	0.1	1991	Adult	Burger *et al*., 1992a
	New York, USA	1.4	1991	Juvenile	Burger and Gochfeld, 1992a
Sooty tern ***Sterna fuscata***	Puerto Rico	0.78	1990	Adult	Gochfeld *et al*., 1991
	Johnston Atoll, Pacific	1.38	1990	Adult	Burger *et al*., 1992c
	Hawaii	2.71	1990	Adult	Burger *et al*., 1992c
	Midway Atoll, Pacific	0.519	1997	Adult	Burger and Gochfeld, 2000a
Roseate tern ***Sterna dougallii***	New York, USA	1.5	1991	Adult	Burger *et al*., 1992a
Grey-backed tern ***Sterna lunata***	Midway Atoll, Pacific	0.942	1997	Adult	Burger and Gochfeld, 2000a
White tern ***Gygis alba***	Midway Atoll, Pacific	1.38	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	0.947	1997	Juvenile	Burger and Gochfeld, 2000a
Black skimmer ***Rynchops niger***	New York, USA	1.39	1991	Adult	Burger and Gochfeld, 1992b
	New York, USA	2.7	1990	Adult	Gochfeld *et al*., 1991

Table 6-6 cont.

Species	Location	Mean concentration ppm (dry weight)	Study year	Notes	Reference
Brown noddy ***Anous stolidus***	Hawaii	1.96	1990	Adult	Burger *et al*., 1992c
	Johnston Atoll, Pacific	1.75	1990	Adult	Burger *et al*., 1992c
	Hawaii	1.9	1990	Adult	Burger, 1993b
	Hawaii	0.88	1990	Juvenile	Burger, 1993b
	Midway Atoll, Pacific	0.289	1997	Adult	Burger and Gochfeld, 2000a
Bonin petrel ***Pterodroma hypoleuca***	Midway Atoll, Pacific	1.35	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	0.802	1997	Juvenile	Burger and Gochfeld, 2000a
Christmas shearwater ***Puffinus nativitatis***	Midway Atoll, Pacific	2.38	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	1.29	1997	Juvenile	Burger and Gochfeld, 2000a
Wedge-tailed shearwater ***Puffinus pacificus***	Hawaii	1.6	1990	Adult	Burger *et al*., 1992c
	Johnston Atoll, Pacific	2.75	1990	Adult	Burger *et al*., 1992c
	Midway Atoll, Pacific	0.478	1997	Adult	Burger and Gochfeld, 2000a
Laysan albatross ***Diomedea immutabilis***	Midway Atoll, Pacific	0.799	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	0.734	1997	Juvenile	Burger and Gochfeld, 2000a
Black-footed albatross ***Diomedea nigripes***	Midway Atoll, Pacific	0.973	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	1.11	1997	Juvenile	Burger and Gochfeld, 2000a
Brown booby ***Sula leucogaster***	Johnston Atoll, Pacific	2.33	1990	Adult	Burger *et al*., 1992c
Red-footed booby ***Sula sula***	Johnston Atoll, Pacific	2.08	1990	Adult	Burger *et al*., 1992c
	Midway Atoll, Pacific	0.975	1997	Adult	Burger and Gochfeld, 2000a
Red-tailed tropicbird ***Phaethon rubricauda***	Midway Atoll, Pacific	0.684	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	0.637	1997	Juvenile	Burger and Gochfeld, 2000a
Great frigatebird ***Fregata minor***	Midway Atoll, Pacific	1.5	1997	Adult	Burger and Gochfeld, 2000a
Osprey ***Pandion haliaetus***	California, USA	0.87	1992-1996	Adult	Cahill *et al*., 1998
Osprey ***Pandion haliaetus***	Florida, USA	0.802	200-2001	Adult	Lounsbury-Billie *et al*., 2008
Laggar falcon ***Falco biarmicus jugger***	Pakistan	1.56	1996	Adult	Movalli, 2000
Gentoo penguin ***Pygoscelis papua***	Antarctica	1.7	2002	Adult	Metcheva *et al*., 2006
	Antarctica	1.57	2003	Adult	Metcheva *et al*., 2006
Chinstrap penguin ***Pygoscelis antarctica***	Antarctica	1.8	2002	Adult	Metcheva *et al*., 2006
	Antarctica	1.66	2003	Adult	Metcheva *et al*., 2006

Table 6-7 Summary of literature data for manganese concentrations in seabird feathers

Species	Location	Mean concentration ppm (dry weight)	Study year	Notes	Reference
Herring gull ***Larus argenatatus***	Northeast Siberia	2.35	1993	Adult	Kim *et al*., 1996
Franklin's gull ***Larus pipixcan***	Interior North America	8.108	1994	Adult	Burger, 1996
	Interior North America	6.827	1994	Juvenile	Burger, 1996
	Minnesota, USA	3.73	1994	Adult	Burger and Gochfeld, 1999
	Minnesota, USA	3.42	1994	Juvenile	Burger and Gochfeld, 1999
Laughing gull ***Larus atricilla***	New York, USA	3.444	1992	Adult male	Gochfeld *et al*., 1996
	New York, USA	4.649	1992	Adult female	Gochfeld *et al*., 1996
Black-tailed gull ***Larus crassirostris***	Japan	0.32	1999-2001	Adult	Agusa *et al*., 2005
Glaucous gull ***Larus hyperboreus***	Northeast Siberia	1.98	1993	Adult	Kim *et al*., 1996
Sabine's gull ***Xema sabin***	Northeast Siberia	2.32	1993	Adult	Kim *et al*., 1996
Parasitic jaeger ***Stercorarius parasiticus***	Northeast Siberia	4.4	1993	Adult	Kim *et al*., 1996
Long-tailed jaeger ***Stercorarius longicaudus***	Northeast Siberia	4.2	1993	Adult	Kim *et al*., 1996
Sooty tern ***Sterna fuscata***	Midway Atoll, Pacific	0.3	1997	Adult	Burger and Gochfeld, 2000a
Grey-backed tern ***Sterna lunata***	Midway Atoll, Pacific	1.12	1997	Adult	Burger and Gochfeld, 2000a
White tern ***Gygis alba***	Midway Atoll, Pacific	0.41	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	1.19	1997	Juvenile	Burger and Gochfeld, 2000a
Arctic tern ***Sterna paradisaea***	Northeast Siberia	5.1	1993	Adult	Kim *et al*., 1996
Black skimmer ***Rynchops niger***	New York, USA	2.5	1991	Adult	Burger and Gochfeld, 1992a
Brown noddy ***Anous stolidus***	Midway Atoll, Pacific	0.424	1997	Adult	Burger and Gochfeld, 2000a
Bonin petrel ***Pterodroma hypoleuca***	Midway Atoll, Pacific	0.561	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	1.14	1997	Juvenile	Burger and Gochfeld, 2000a
Christmas shearwater ***Puffinus nativitatis***	Midway Atoll, Pacific	2.05	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	2.07	1997	Juvenile	Burger and Gochfeld, 2000a
Wedge-tailed shearwater ***Puffinus pacificus***	Midway Atoll, Pacific	0.718	1997	Adult	Burger and Gochfeld, 2000a
Laysan albatross ***Diomedea immutabilis***	Midway Atoll, Pacific	1.72	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	1.63	1997	Juvenile	Burger and Gochfeld, 2000a
Black-footed albatross ***Diomedea nigripes***	Midway Atoll, Pacific	1.78	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	2.03	1997	Juvenile	Burger and Gochfeld, 2000a
Red-footed booby ***Sula sula***	Midway Atoll, Pacific	1.46	1997	Adult	Burger and Gochfeld, 2000a
Red-tailed tropicbird ***Phaethon rubricauda***	Midway Atoll, Pacific	0.678	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	0.603	1997	Juvenile	Burger and Gochfeld, 2000a
Great frigatebird ***Fregata minor***	Midway Atoll, Pacific	0.59	1997	Adult	Burger and Gochfeld, 2000a
Arctic loon ***Gavia arctica***	Northeast Siberia	4.68	1993	Adult	Kim *et al*., 1996
Gentoo penguin ***Pygoscelis papua***	Antarctica	1.5	2002	Adult	Metcheva *et al*., 2006
	Antarctica	2.6	2003	Adult	Metcheva *et al*., 2006
Chinstrap penguin ***Pygoscelis antarctica***	Antarctica	1.4	2002	Adult	Metcheva *et al*., 2006
	Antarctica	1.6	2003	Adult	Metcheva *et al*., 2006

Table 6-8 Summary of literature data for nickel concentrations in seabird feathers

Species	Location	Mean concentration ppm (dry weight)	Study year	Notes	Reference
Osprey ***Pandion haliaetus***	Florida, USA	0.967	200-2001	Adult	Lounsbury-Billie *et al*., 2008
Gentoo penguin ***Pygoscelis papua***	Antarctica	0.84	2002	Adult	Metcheva *et al*., 2006
	Antarctica	2.2	2003	Adult	Metcheva *et al*., 2006
Chinstrap penguin ***Pygoscelis antarctica***	Antarctica	0.65	2002	Adult	Metcheva *et al*., 2006
	Antarctica	0.75	2003	Adult	Metcheva *et al*., 2006

Table 6-9 Summary of literature data for selenium concentrations in seabird feathers

Species	Location	Mean concentration ppm (dry weight)	Study year	Notes	Reference
Franklin's gull ***Larus pipixcan***	Interior North America	2.040	1994	Adult	Burger, 1996
	Interior North America	0.965	1994	Juvenile	Burger, 1996
	Minnesota, USA	1.56	1994	Adult	Burger and Gochfeld, 1999
	Minnesota, USA	0.927	1994	Juvenile	Burger and Gochfeld, 1999
Laughing gull ***Larus atricilla***	New York, USA	1.437	1992	Adult male	Gochfeld *et al*., 1996
	New York, USA	1.859	1992	Adult female	Gochfeld *et al*., 1996
Black-tailed gull ***Larus crassirostris***	Japan	1.1	1999-2001	Adult	Agusa *et al*., 2005
Common tern ***Sterna hirundo***	New York, USA	2.1	1991	Adult	Burger *et al*., 1992a
	Massachusesetts, USA	2	1991	Adult	Burger *et al*., 1992a
	New York, USA	1.2	1991	Juvenile	Burger and Gochfeld, 1992a
Sooty tern ***Sterna fuscata***	Hawaii	4.4	1990	Adult	Burger *et al*., 1992c
	Johnston Atoll	3.79	1990	Adult	Burger *et al*., 1992c
	Midway Atoll, Pacific	3.42	1997	Adult	Burger and Gochfeld, 2000a
Roseate tern ***Sterna dougallii***	New York, USA	3.900	1991	Adult	Burger *et al*., 1992a
Grey-backed tern ***Sterna lunata***	Midway Atoll, Pacific	2.87	1997	Adult	Burger and Gochfeld, 2000a
White tern ***Gygis alba***	Midway Atoll, Pacific	1.29	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	0.63	1997	Juvenile	Burger and Gochfeld, 2000a
Black skimmer ***Rynchops niger***	New York, USA	1.2	1991	Adult	Burger and Gochfeld, 1992b
Brown noddy ***Anous stolidus***	Hawaii	7.59	1990	Adult	Burger *et al*., 1992c
	Johnston Atoll	11.15	1990	Adult	Burger *et al*., 1992c
	Hawaii	7.5	1990	Adult	Burger, 1993b
	Hawaii	1.4	1990	Juvenile	Burger, 1993b
	Midway Atoll, Pacific	3.99	1997	Adult	Burger and Gochfeld, 2000a
Bonin petrel ***Pterodroma hypoleuca***	Midway Atoll, Pacific	7.85	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	4.86	1997	Juvenile	Burger and Gochfeld, 2000a

Table 6-9 cont.

Species	Location	Mean concentration ppm (dry weight)	Study year	Notes	Reference
Christmas shearwater ***Puffinus nativitatis***	Midway Atoll, Pacific	10.1	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	4.98	1997	Juvenile	Burger and Gochfeld, 2000a
Wedge-tailed shearwater ***Puffinus pacificus***	Hawaii	3.23	1990	Adult	Burger *et al*., 1992c
	Johnston Atoll	4.06	1990	Adult	Burger *et al*., 1992c
	Midway Atoll, Pacific	4.06	1997	Adult	Burger and Gochfeld, 2000a
Laysan albatross ***Diomedea immutabilis***	Midway Atoll, Pacific	2.29	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	1.7	1997	Juvenile	Burger and Gochfeld, 2000a
Black-footed albatross ***Diomedea nigripes***	Midway Atoll, Pacific	3.26	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	2.33	1997	Juvenile	Burger and Gochfeld, 2000a
Brown booby ***Sula leucogaster***	Johnston Atoll	3.69	1990	Adult	Burger *et al*., 1992c
Red-footed booby ***Sula sula***	Johnston Atoll	2.28	1990	Adult	Burger *et al*., 1992c
	Midway Atoll, Pacific	2.34	1997	Adult	Burger and Gochfeld, 2000a
Red-tailed tropicbird ***Phaethon rubricauda***	Midway Atoll, Pacific	3.99	1997	Adult	Burger and Gochfeld, 2000a
	Midway Atoll, Pacific	3.94	1997	Juvenile	Burger and Gochfeld, 2000a
Great frigatebird ***Fregata minor***	Midway Atoll, Pacific	4.54	1997	Adult	Burger and Gochfeld, 2000a
Osprey ***Pandion haliaetus***	California, USA	3.2	1992-1996	Adult	Cahill *et al*., 1998
Laggar falcon ***Falco biarmicus jugger***	Pakistan	2.76	1996	Adult	Movalli, 2000
Gentoo penguin **Pygoscelis papua**	Antarctica	2	2002	Adult	Metcheva *et al*., 2006
	Antarctica	1.8	2003	Adult	Metcheva *et al*., 2006
Chinstrap penguin ***Pygoscelis antarctica***	Antarctica	<0.80	2002	Adult	Metcheva *et al*., 2006
	Antarctica	<0.80	2003	Adult	Metcheva *et al*., 2006

Table 6-10 Summary of literature data for vanadium concentrations in seabird feathers

Species	Location	Mean concentration ppm (dry weight)	Study year	Notes	Reference
Black-tailed Gull ***Larus crassirostris***	Japan	0.076	1999-2001	Adult	Agusa *et al*., 2005
Osprey ***Pandion haliaetus***	Florida, USA	1.06	200-2001	Adult	Lounsbury-Billie *et al*., 2008

Table 6-11 Summary of literature data for zinc concentrations in seabird feathers

Species	Location	Mean concentration ppm (dry weight)	Study year	Notes	Reference
Herring gull ***Larus argenatatus***	Northeast Siberia	74.5	1993	Adult	Kim *et al*., 1996
Black-tailed Gull ***Larus crassirostris***	Japan	43.9	1999-2001	Adult	Agusa *et al*., 2005
Glaucous gull ***Larus hyperboreus***	Northeast Siberia	78.4	1993	Adult	Kim *et al*., 1996
Sabine's gull ***Xema sabin***	Northeast Siberia	142	1993	Adult	Kim *et al*., 1996
Parasitic jaeger ***Stercorarius parasiticus***	Northeast Siberia	91	1993	Adult	Kim *et al*., 1996
Long-tailed jaeger ***Stercorarius longicaudus***	Northeast Siberia	88.9	1993	Adult	Kim *et al*., 1996
Arctic tern ***Sterna paradisaea***	Northeast Siberia	130	1993	Adult	Kim *et al*., 1996
Arctic loon ***Gavia arctica***	Northeast Siberia	88.4	1993	Adult	Kim *et al*., 1996
Osprey ***Pandion haliaetus***	California, USA	173	1992-1996	Adult	Cahill *et al*., 1998
Laggar falcon ***Falco biarmicus jugger***	Pakistan	107.4	1996	Adult	Movalli, 2000
Gentoo penguin ***Pygoscelis papua***	Antarctica	106	2002	Adult	Metcheva *et al*., 2006
	Antarctica	89	2003	Adult	Metcheva *et al*., 2006
Chinstrap penguin ***Pygoscelis antarctica***	Antarctica	99	2002	Adult	Metcheva *et al*., 2006
	Antarctica	75	2003	Adult	Metcheva *et al*., 2006

6.1.1 Lead and cadmium in feathers

Gochfeld *et al*. (1996) examined concentrations of lead and cadmium in the feathers (and other tissues) of laughing gulls (*L. atricilla*). The overall conclusion of this study was that feathers gave an indication of concentrations of lead in other tissues, but, conversely, no significant correlation was found between cadmium in feathers and concentrations in other tissues. Dauwe *et al*. (2004) determined concentrations of metals in feathers of great tits (*P. major*) and compared these with metal concentrations in food samples. Lead concentrations in feathers were found to be significantly positively correlated with concentrations in the great tits' main invertebrate food source. No significant relationship was found for cadmium. These results were consistent with those of an earlier study investigating the potential use of excrement and feathers of nestling great (*P. major*) and blue tits (*Parus caeruleus*) to reflect internal heavy metal contamination (Dauwe *et al.*, 2000). A significant correlation was found between the lead concentration in the excrement and feathers for great tits ($p < 0.0005$). A significant correlation was also found between excrement and feathers for blue tits ($p < 0.05$), although this correlation was strongly influenced by a single point and when this point was removed the correlation was no longer significant ($p > 0.1$). No significant correlation was found for cadmium for either of the species studied. These results indicate that lead accumulates in feathers, and that feathers may be suitable as a biomonitor for lead pollution.

For cadmium, there is controversy surrounding whether feathers actually accumulate it; some authors have found high concentrations (Mayack *et al.*, 1981) while others have not (Osborn *et al.*, 1979). Information on cadmium concentrations in seabird feathers is conflicting, and whilst some authors suggest that there is little evidence of a relationship to other tissue concentrations (Osborn *et al.*, 1979; Howarth *et al.*, 1981; Dauwe *et al.*, 2000; Dauwe *et al.*, 2004), some studies have demonstrated a significant relationship between cadmium concentrations in feathers and those in other tissues. For example, Battaglia *et al*. (2005) investigated lead and cadmium concentrations in the common buzzard (*B. buteo*) and little owl (*A. noctua*), using liver, kidneys, pectoral muscle, sternum bone and feathers. Significant correlations were observed for cadmium between liver and feathers and between kidney and feathers in buzzards, and between kidney and feathers in little owls, indicating that cadmium accumulated from the diet could be excreted through the feathers. For lead, a significant correlation was found between bone and feathers in little owls; however, no relationship was found between feathers and any other tissues for lead in buzzards.

Interpretation of both lead and cadmium concentrations in feathers is difficult as there is evidence of heavy contamination by secretory products and atmospheric deposition. Concentrations of lead have been found to be higher in feathers than other tissues (Gochfeld *et al.*, 1996), and it has been proposed that the concentration of lead in feathers is a good predictor of internal dose (Burger, 1993; Burger & Gochfeld, 2004). However, a number of authors suggest that the majority of cadmium and lead originates from direct atmospheric deposition onto feather surfaces, with ingested cadmium and lead becoming firmly bound in kidney and bone, only entering feathers in trace amounts (Goede & de Bruin, 1986; Walsh, 1990; Furness *et al.*, 1993; Stewart *et al.*, 1994). Experimental studies with starlings (*Sturnus vulgaris*; Pilastro *et al.*, 1993) and zebra finches (*Taeniopygia guttata*; Dauwe *et al.*, 2002b) have also demonstrated that lead and cadmium are deposited onto the surface of the feather in secretions during preening. Thus, whilst reported concentrations of lead and cadmium illustrate the use of feathers as a general monitor of metal contamination, if the majority of lead and cadmium in feathers originates from external exposure, the results say little about food chain contamination. As previously mentioned, washing of feathers has been carried out in some studies in an attempt to remove surface deposits of heavy metals (Goede & de Bruin, 1986); however the effectiveness of this for lead and cadmium is yet to be demonstrated and some authors have demonstrated experimentally that the exogenous fraction of both cadmium and lead cannot be completely removed by washing procedures (Weyers *et al.*, 1988). Another way to minimise the effects of exogenous contamination is to use feathers (down) of nestlings. Young nestlings

will have had little exposure to the environment, meaning that exogenous contamination onto the feather surface is limited and the growing feathers of nestling birds may thus represent the body load of contaminants better than those of adults (Dauwe *et al.*, 2004).

6.1.2 Other metals in feathers

There has been very little research investigating the potential use of feathers as indicators for non-essential metals other than mercury, lead and cadmium, and the limited results are conflicting. However, although not related back to tissue concentrations, concentrations of heavy metals and selenium in feathers have been measured in a number of field studies (see Tables 6-1 to 6-11, above).

Burger (1993) reviews the ratios of metal concentrations in other tissues to concentrations in feathers of gull species reported in a number of studies. Table 6-12 summarises some of this data.

Table 6-12 Ratio of metal concentrations in other tissues to concentrations in feathers for gull species (after Burger, 1993a)

Metal	Liver	Kidney	Muscle	Bone	Brain
Cadmium	2.75	8.67	0.53	0.96	0.31
Iron	25.6	4.18	1.99	0.48	0.07
Lead	0.42	0.33	0.14	3.72	0.13
Manganese	1.17	0.69	0.09	2.92	0.13
Nickel	0.25	0.2	0.23	0.29	0.11
Selenium	1.2	2.2	0.4	0.3	0.25
Zinc	0.38	0.39	0.24	1.16	-

Gochfeld *et al*. (1996) examined the tissue relationships between feather and other tissues in laughing gulls (*L. atricilla*) and found similar ratios to those described in other studies (as summarised in Burger, 1993a) for lead, mercury, selenium and manganese, but not for cadmium. This suggests that the concentrations of some metals in feathers can be used to predict the relative concentrations in other tissues.

In a study investigating the potential use of excrement and feathers of nestling great tits (*P. major*) and blue tits (*P. caeruleus*) to reflect internal heavy metal contamination, Dauwe *et al*. (2000) found no significant correlation between the arsenic concentration in the excrement and feathers of the birds studied. However, in a later study comparing concentrations of arsenic in feathers of great tits (*P. major*) with concentrations in food samples, Dauwe *et al*. (2004) found

arsenic concentrations in feathers to be significantly positively correlated with concentrations in the great tits' main invertebrate food source. A significant feather/liver correlation of arsenic concentrations has also been noted for estuarine gulls in New Zealand (Turner *et al.*, 1978). As with lead and cadmium, arsenic concentrations can originate from exogenous deposition as well as endogenous (Goede & de Bruin, 1984).

Dauwe *et al*. (2000) found no significant correlation between zinc or copper concentrations in the excrement and feathers of great tits (*P. major*) and blue tits (*P. caeruleus*). The same authors report no relationship between concentrations of copper, nickel and zinc in the feathers of great tits and concentrations in food samples (Dauwe *et al.*, 2004). It has been suggested that the accumulation of essential trace elements such as zinc, manganese, copper and iron (the latter of which is required in large amounts for feather formation) may be regulated in the body to keep concentrations in internal tissues physiologically adequate (Clarkson, 1986; Burger, 1993), and indeed the above studies would suggest that feathers may not be suitable as a biomonitor for these metals. However, other studies have shown that this is not the case (Goede & de Bruin, 1986; Burger, 1993; Burger & Gochfeld, 1993; Gochfeld *et al.*, 1996). It has been reported that 30-40% of the body burden of copper is sequestered into feathers (Burger, 1993), and nickel has been shown to be sequestered in the feathers of dietary-exposed birds (Eastin & O'Shea, 1981). Goede and de Bruin (1986) found that, for zinc, external contamination of the feather did not appear to be occurring, and thus the concentrations of zinc found in feathers could be said to reflect internal concentrations. Detectable concentrations of selenium have been reported in feathers (Stoneburner *et al.*, 1980; Burger & Gochfeld, 1992a; Burger & Gochfeld, 1992b), but contamination of feathers with selenium via excretions from the uropygial gland during preening (Goede & de Bruin, 1984) can confound assessments of the degree to which selenium is sequestered into feathers as a result of internal tissue burdens.

Clearly there is scope for more investigation into the use of feathers as biomonitors for metals other than mercury, lead and cadmium.

6.2 Methods

Samples of down feathers were obtained from chicks less than 48 hours post-hatching at Lymington and Poole. Down of newly hatched chicks was sampled as the chicks will have had little exposure to the environment, thus exogenous contamination limited and feathers represent

contamination acquired almost entirely on the breeding grounds and from development in the egg.

Chicks less than 48 hours old were identified by being largely immobile (black-headed gull chicks are semi-altricial and largely immobile after hatching (see Section 1.5)), and sampling was also carried out on single chicks sharing a nest with eggs, as the entire clutch hatches over a 48 hour period (Eising *et al.*, 2001) and thus a chick in a nest with unhatched eggs is likely to be less than 48 hours old. As down was cut from chicks rather than plucked, this non-invasive sampling method was included on and thus covered by the English Nature/Natural England license mentioned above (Section 2.2.1). Ten chicks were chosen at random from each site, with only one chick per nest being sampled. A pair of small dissecting scissors was rinsed with acetone prior to each new chick to be sampled, in order to minimise any potential contamination. Down was snipped from the body of each chick (the general torso area) and placed in sealable polythene bags; the chick was then replaced in the nest where it was found.

Down was washed alternately with Milli-Q® water and 1 mol/l Aristar grade acetone to remove any loosely adhered external contamination (Appelquist *et al.*, 1984; Walsh, 1990; Burger, 1993), and then air dried. A fresh weight was then obtained from the air-dry feathers, which were then placed in an oven at 60ºC and dried to a constant weight. Samples were weighed into microwave digestion vessels and 5 ml of concentrated nitric acid added. Contamination was minimised at all stages by acid-washing all glassware prior to rinsing with Milli-Q® water, and rinsing any metal equipment with ultra high purity (BDH Chemicals ARISTAR®) grade acetone before bringing it into contact with samples. In addition, all procedures were carried out in a laminar-flow cabinet.

Samples of down were analysed individually for arsenic, cadmium, cobalt, copper, iron, lead, manganese, nickel, selenium, vanadium and zinc. The microwave digestion and analytical method (USEPA Method 3051, analysis by ICP-MS) used for the down samples was as described for the egg samples in Chapter 5, Section 5.2. Again, with each batch of samples determined, a calibration graph was constructed to check for linearity and to calculate concentrations in the samples, and blank samples of 2% nitric acid were analysed to assess contamination. Measured samples were blank-corrected to provide reportable data.

To determine if any bias exists in the environmental media compared with the Milli-Q® water calibrant samples (used to construct a calibration curve and hence calculate concentrations), spike-and-recovery tests were carried out for feather samples, achieved by spiking samples with

varying concentrations of metal standards and constructing individual calibration curves. Accordingly, all calculations of metal concentrations in the feathers were calculated using slopes derived from calibrations established in the same biological matrix.

6.2.1 Results of initial trials

Initial trials were carried out in order to assess losses made during the sample preparation method. Trials were carried out for all the metals investigated in this study.

Washed vs unwashed feathers

Prior to carrying out the spike-recovery trials for the feathers, a trial was carried out in order to assess the effectiveness of washing the feathers to remove external contamination. Duck feathers were collected, taking care to use downy feathers in the trial in order to be as representative of the gull feather samples as possible. The washing procedure was as follows: feathers were washed once with acetone and then rinsed three times with Milli-Q® water. This method of washing feathers with acetone and distilled water to remove external contamination has been used by a number of authors (Burger *et al.*, 1993; Burger, 1996; Gochfeld *et al.*, 1996; Burger & Gochfeld, 1997; Burger & Gochfeld, 1999; Eens *et al.*, 1999; Burger & Gochfeld, 2000a; Burger & Gochfeld, 2000b; Dauwe *et al.*, 2000; Janssens *et al.*, 2001; Janssens *et al.*, 2002; Dauwe *et al.*, 2003; Dauwe *et al.*, 2004; Dauwe *et al.*, 2005).

Each of six duck feather samples were divided into two, with one half of the sample to be analysed unwashed, and the other half to be washed as described above, prior to analysis. Feathers were placed into pre-weighed jars and the 'unwashed' feather samples weighed to obtain the fresh weight. Jars were then covered with a nylon mesh secured with an elastic band at the neck of the jar. Acetone was poured through the mesh of the 'washed' feather samples and the contents shaken for a few minutes. The acetone was then poured out of the jar through the mesh, allowing for the acetone and any small particles of debris to be removed from the jar, whilst leaving the feathers inside. The procedure was then repeated three times using Milli-Q® water rather than acetone. The washed feathers were then left to air dry, after which they were weighed to obtain the fresh weight. All samples were then placed in an oven at 60ºC overnight, and the dry weight obtained. Finally, the dried samples were weighed into microwave extraction vessels, 5 ml of concentrated nitric acid was added, and microwave digestion carried

out following the procedure outlined in Chapter 5, Section 5.2. Analysis was carried out using ICP-MS.

Data for the metal concentrations measured in this trial are provided in Appendix D. The data show that, for a number of metals, the concentrations measured in the washed feathers were considerably lower than for the unwashed feathers. This was the case for arsenic, cobalt, iron, manganese, nickel, lead and vanadium, indicating that the washing procedure is useful for removing external contamination which could contain these metals. Of the remaining metals, copper, selenium and zinc concentrations were also lower in washed feathers than unwashed, although the differences in concentrations were not as great as for the metals mentioned above. Cadmium concentrations were low in both sets of samples, although very slightly lower in the washed samples than the unwashed.

Overall, the trial would suggest that washing feather samples using the method described above is effective in removing at least some of the external contamination, which would likely lead to the measurement of inaccurately high concentrations of metals in the samples, particularly for arsenic, cobalt, iron, manganese, nickel, lead and vanadium. The washing procedure outlined above was therefore employed prior to digestion and analysis of the gull feather samples in this study.

Multi-element trials

As for the previous test, duck feathers were collected and downy feathers used in order to be as representative of the gull feather samples as possible. Each feather sample was divided approximately in half and the separate samples placed into clean acid-washed, pre-weighed glass jars. A thin nylon mesh was then placed over the opening of each jar and secured with an elastic band around the neck. This enabled the feathers to be washed without any loss of sample. Each jar was then rinsed through with acetone, followed by three further rinses with Milli-Q® water, and then left to air dry. The jars were then weighed and the sample weight calculated. Of the two samples from each feather, one was spiked with a known concentration of a standard solution of metals (as used in the egg multi-element trials; Section 5.2.1) and the corresponding sample was left unspiked. This was repeated with varying concentrations of metals. All samples were then placed in an oven at 60ºC and left to dry to a constant mass, after which they were re-weighed to obtain the dry weight and enable calculation of the water content. The dried contents of each jar were then accurately weighed into microwave extraction

vessels. The samples were microwave digested following the procedure outlined in Chapter 5, Section 5.2, after which analysis was carried out.

Feathers were spiked with varying amounts of a multi-element standard (Fisher Scientific, WP-15) containing 100 mg/l arsenic, copper, iron, manganese, nickel, lead and zinc, 25 mg/l cadmium and selenium and 250 mg/l vanadium, in a matrix of 5 % nitric acid. Feather 1 was spiked with 0.1 ml of this standard, Feather 2 with 0.5 ml, Feather 3 samples with 1 ml, Feather 4 samples with 2.5 ml and Feather 5 samples with 5 ml.

Table 6-13 provides average recoveries for each metal.

Table 6-13 Mean recoveries for metals in duck feather samples

Metal	Recovery range (%)	Mean recovery (%)
As	54.6 - 90.9	71.7
Cd	58.9 - 99.3	77.4
Co	55.1 - 92.3	74.9
Cu	59.8 - 97.8	77.8
Fe	58.2 - 99.0	80.4
Pb	64.8 - 110.0	84.1
Mn	61.3 - 103.2	81.4
Ni	58.5 - 99.4	77.8
Se	53.3 - 87.7	69.3
V	58.7 - 99.5	77.7
Zn	52.6 - 86.9	68.2

Recovery data were good for all metals, with between 68 and 85% average recovery, and the method used in this study is therefore considered suitable for the analysis of the above metals in bird feathers. As with the egg data, analytical results for the black-headed gull feather samples were corrected according to the recovery data. A full set of recovery data with individual concentrations of each metal in feathers is provided in Appendix E.

6.3 Results

Figures 6-1 to 6-11 show mean concentrations (with standard error bars) of each of the metals analysed in feathers from chicks at the Lymington and Poole sites. In addition, a mean concentration calculated from values reported in the literature for juvenile seabird feathers on a

global scale is presented (for actual concentrations reported in the literature, see Section 6.1, Tables 6-1 to 6-11).

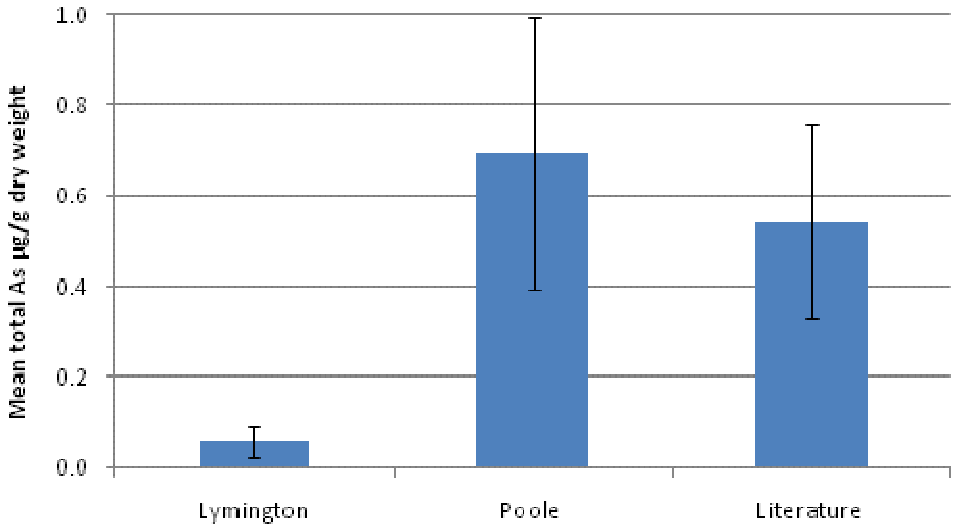


Figure 6-1 **Mean arsenic concentrations (µg/g dry weight), ± standard error, in black-headed gull chick down from Lymington and Poole colonies (N = 24), compared with literature data for juvenile seabird feathers (N = 7)**

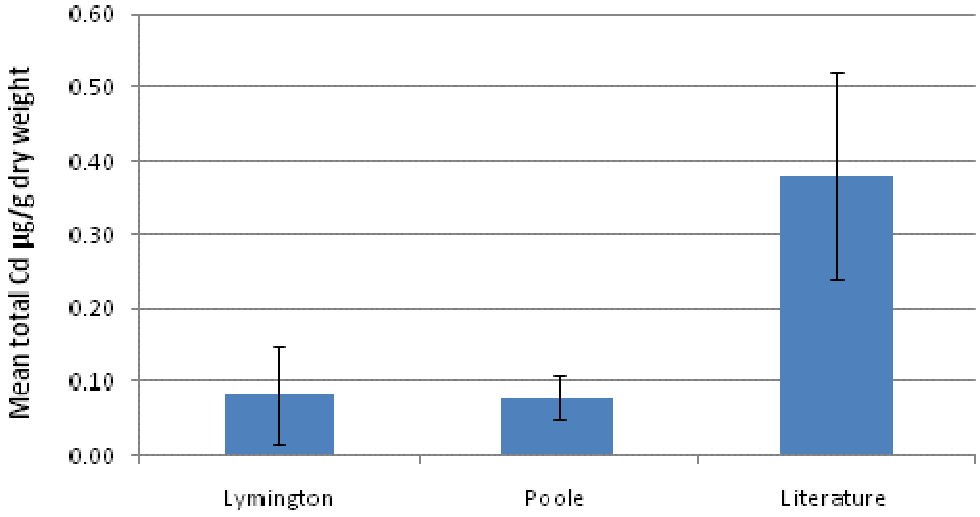


Figure 6-2 **Mean cadmium concentrations (µg/g dry weight), ± standard error, in black-headed gull chick down from Lymington and Poole colonies (N = 24), compared with literature data for juvenile seabird feathers (N = 10)**

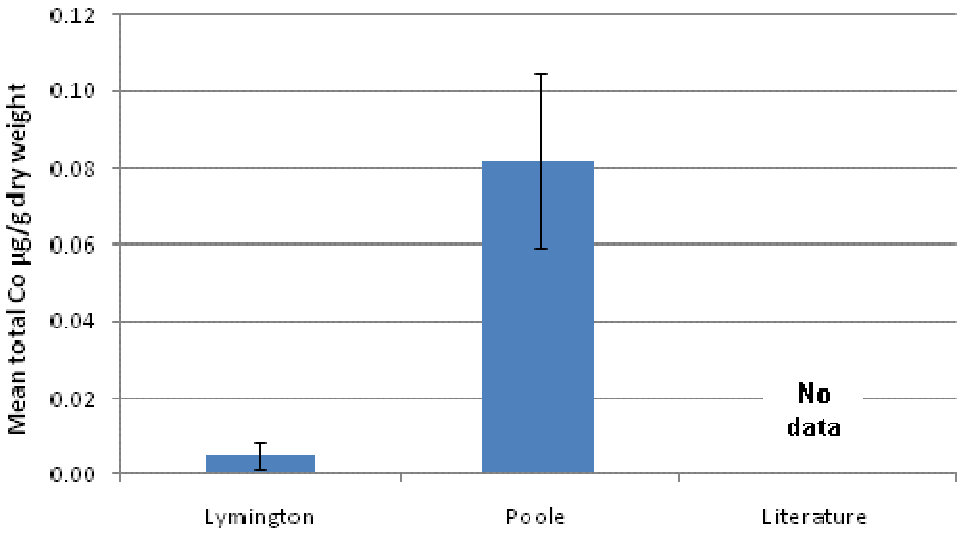


Figure 6-3 **Mean cobalt concentrations (µg/g dry weight), ± standard error, in black-headed gull chick down from Lymington and Poole colonies (N = 24)**

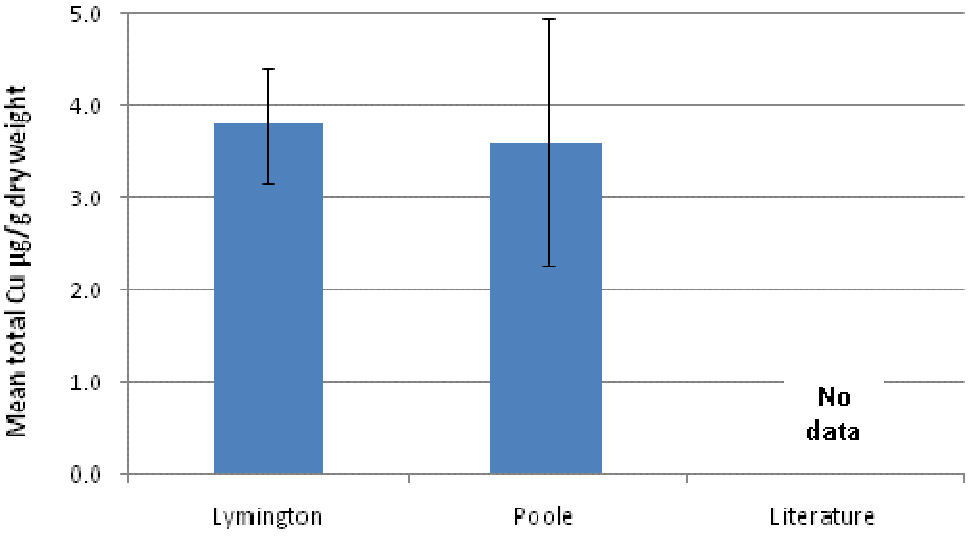


Figure 6-4 **Mean copper concentrations (µg/g dry weight), ± standard error, in black-headed gull chick down from Lymington and Poole colonies (N = 24)**

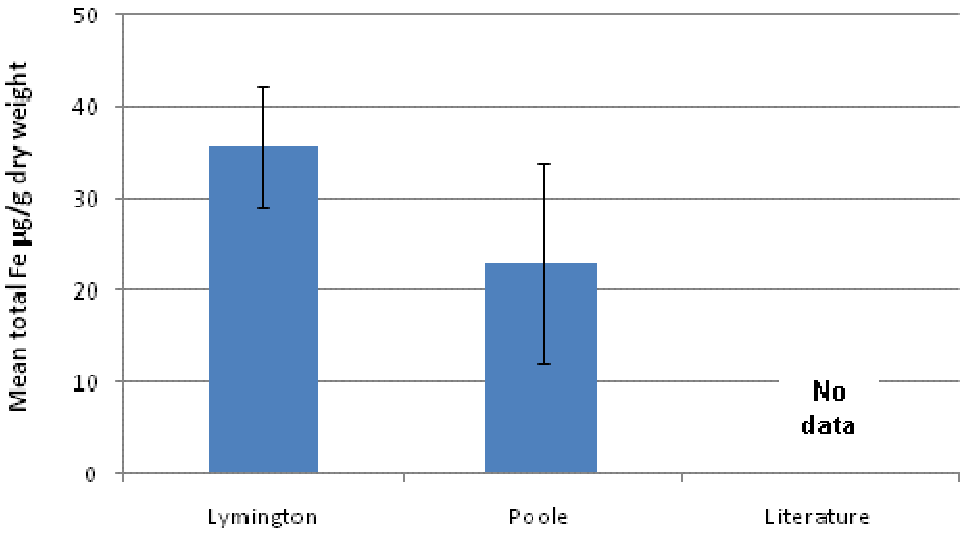


Figure 6-5 **Mean iron concentrations (µg/g dry weight), ± standard error, in black-headed gull chick down from Lymington and Poole colonies (N = 24)**

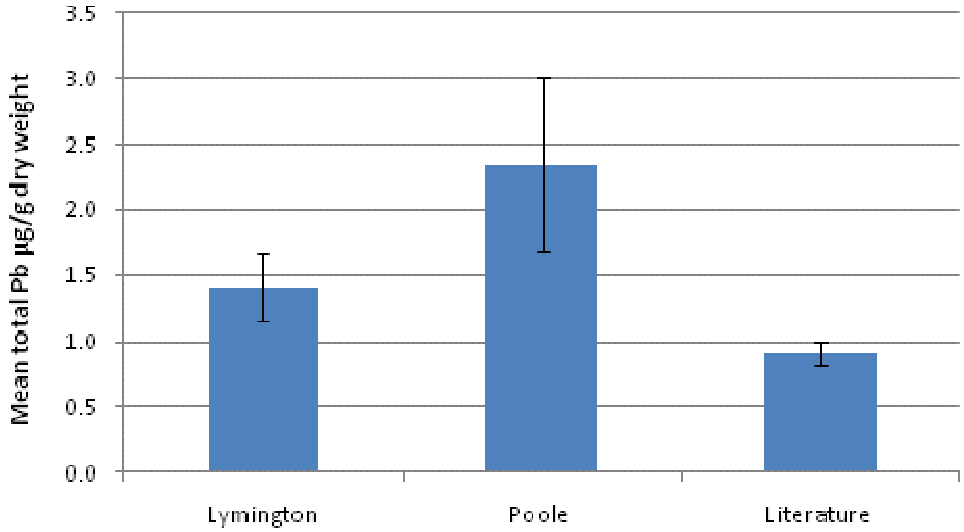


Figure 6-6 **Mean lead concentrations (µg/g dry weight), ± standard error, in black-headed gull chick down from Lymington and Poole colonies (N = 24), compared with literature data for juvenile seabird feathers (N = 11)**

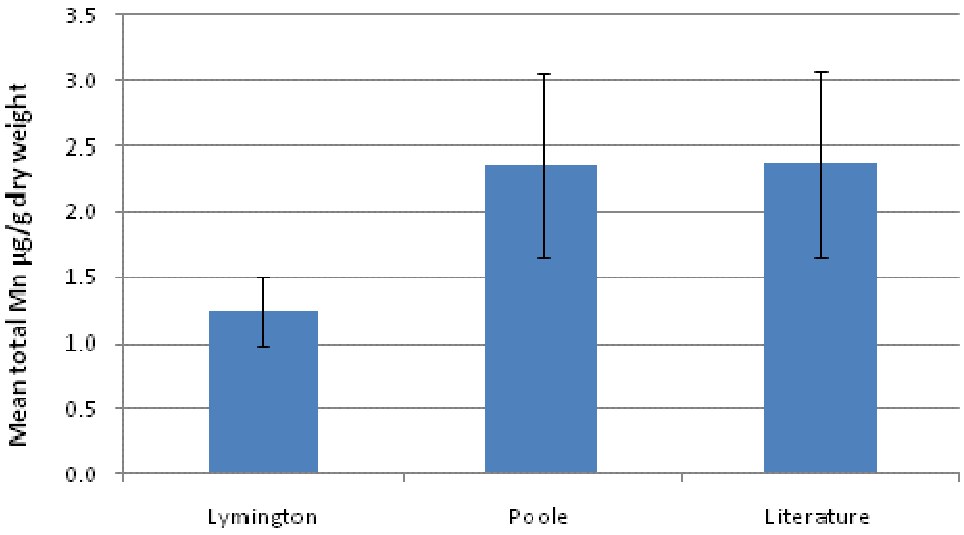


Figure 6-7 **Mean manganese concentrations (µg/g dry weight), ± standard error, in black-headed gull chick down from Lymington and Poole colonies (N = 24), compared with literature data for juvenile seabird feathers (N = 8)**

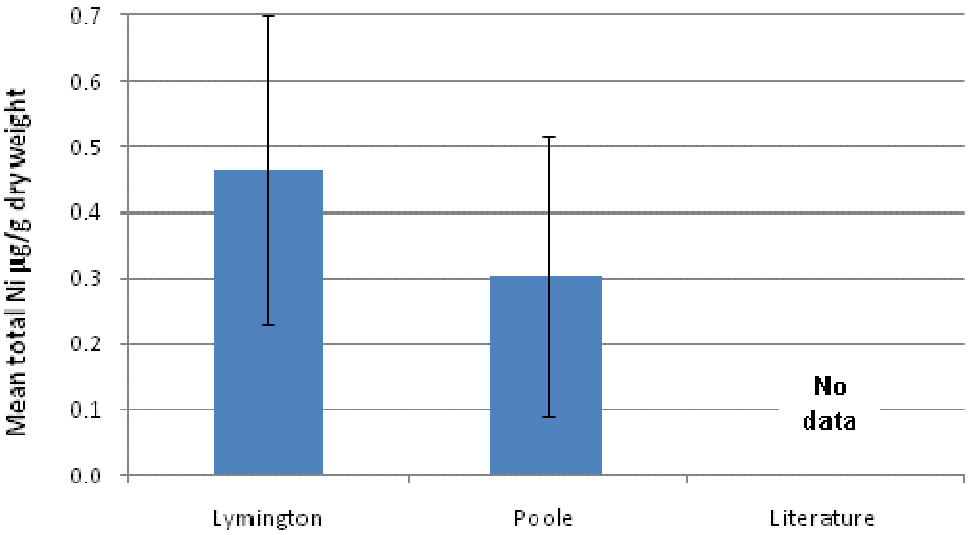


Figure 6-8 **Mean nickel concentrations (µg/g dry weight), ± standard error, in black-headed gull chick down from Lymington and Poole colonies (N = 24)**

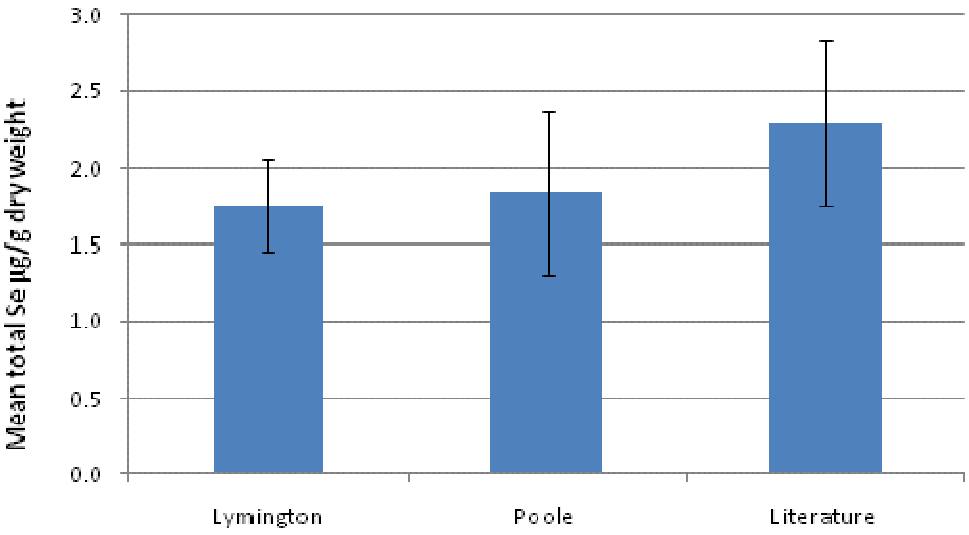


Figure 6-9 **Mean selenium concentrations (µg/g dry weight), ± standard error, in black-headed gull chick down from Lymington and Poole colonies (N = 24), compared with literature data for juvenile seabird feathers (N = 10)**

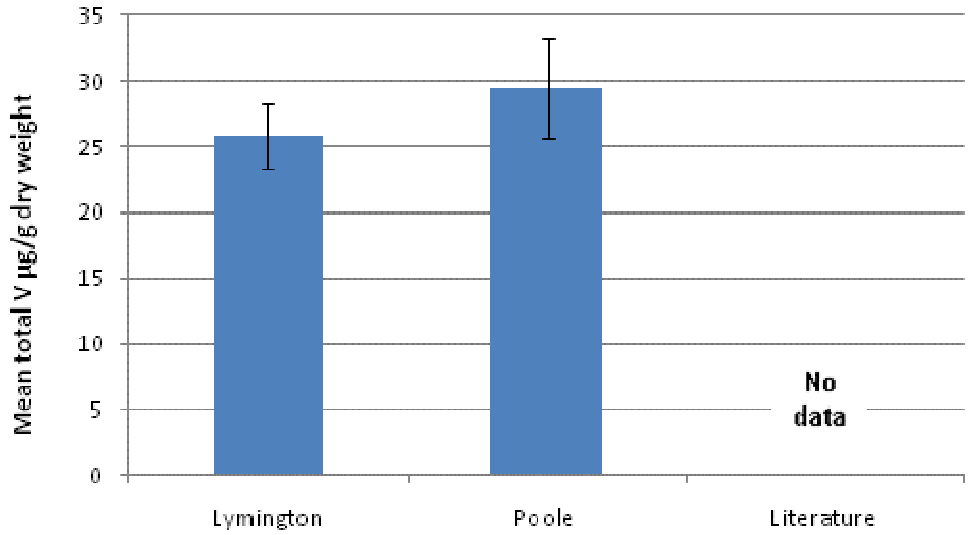


Figure 6-10 **Mean total vanadium concentrations (µg/g dry weight), ± standard error, in black-headed gull chick down from Lymington and Poole colonies (N = 24)**

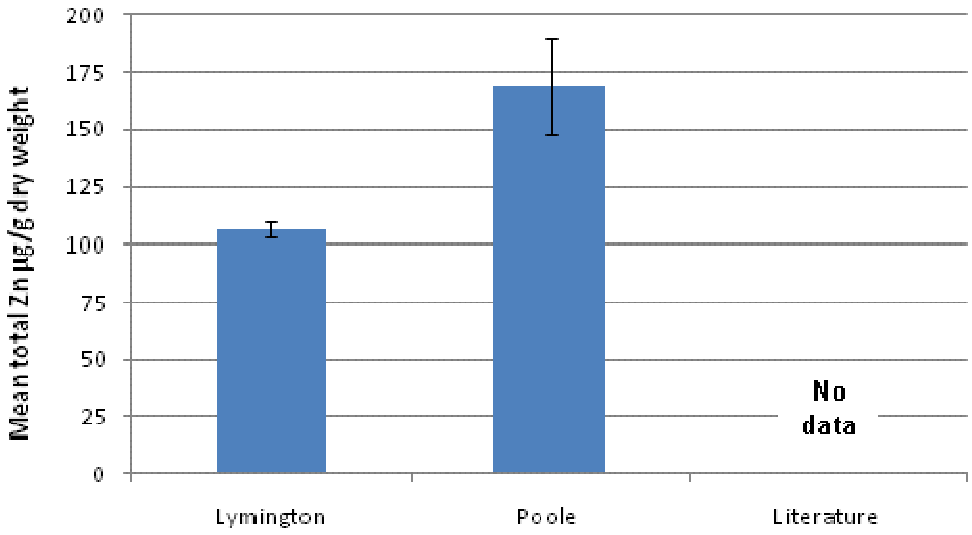


Figure 6-11 Mean total zinc concentrations (µg/g dry weight), ± standard error, in black-headed gull chick down from Lymington and Poole colonies (N = 24)

Statistical analysis

A K-S test was used to check that data were normally distributed, and revealed that data for copper, iron, selenium and vanadium were normally distributed, while distributions of all other metals analysed were not normal. Various transformations were carried out in order to attempt to normalise the data (as detailed in Section 5.3.2). Logarithmic transformation produced a normalised distribution for manganese, lead and zinc. However, data for arsenic, cadmium, cobalt and nickel could not be normalised with any of the transformations attempted. The concentrations of arsenic, cadmium, cobalt and nickel in the down samples were largely below the limit of detection (<0.001 µg/g) and, as can be seen in Figures 6-1, 6-2, 6-3 and 6-8, above, the errors associated with the results for these metals are very large. As the requirements for parametric tests were not met, the results for arsenic, cadmium, cobalt and nickel in chick down must be analysed using non-parametric tests. Variance was examined using Levene's test for equality of variance.

An independent t-test was used to assess whether there were any significant differences between the metal concentrations measured in chick down from the Lymington and Poole sites for copper, iron, lead, manganese, selenium, vanadium and zinc. As the data for arsenic, cadmium, cobalt and nickel were not normally distributed, a non-parametric Mann-Whitney test was used to examine the data. The results of these statistical tests are provided in Table 6-14. Data

provided in the table are based on untransformed data for arsenic, cadmium, cobalt, copper, iron, nickel, selenium and vanadium, and log transformed data for manganese, lead and zinc (data log transformed prior to running independent t-test, and then calculated back to the original scale for the purposes of reporting).

Table 6-14 Test results for differences in metal concentrations in black-headed gull chick down between Lymington and Poole colonies, 2006

Measurement	S.E.	p	Significance
Arsenic	0.162	0.019	*
Cadmium	0.034	0.051	NS
Cobalt	0.014	0.001	**
Copper	1.478	0.901	NS
Iron	12.682	0.327	NS
Lead †	1.251	0.232	NS
Manganese †	1.391	0.602	NS
Nickel	0.156	0.481	NS
Selenium	0.615	0.901	NS
Vanadium	4.517	0.428	NS
Zinc †	1.114	0.003	**

Bold indicates a significant difference between sample sets; ** = highly significant ($p \leq 0.001$); NS = not significant ($p > 0.05$). Data are for independent t-test with the exception of highlighted areas, which indicate results from non-parametric Mann-Whitney test. † data log transformed prior to running test, reported as per original scale.

The test results show that there are significant differences between sample sets for arsenic, cobalt and zinc concentrations in chick down, with down from the chicks on the Poole colony containing significantly higher concentrations of arsenic, cobalt and zinc than down from chicks on the Lymington colony. There are no significant differences between Lymington and Poole feather samples for cadmium, copper, iron, lead, manganese, nickel, selenium or vanadium.

6.4 Discussion

6.4.1 Comparison between sites and with egg concentration data

The statistical tests carried out in Section 6.3 revealed significant differences between the feather samples from the Lymington and Poole sites, with the down of chicks from the Poole site containing significantly greater concentrations than down of chicks from the Lymington site for arsenic (mean concentrations 0.06 μg/g and 0.69 μg/g dry weight, respectively; $p = 0.019$), cobalt (mean concentrations 0.005 μg/g and 0.08 μg/g dry weight, respectively; $p = 0.001$) and zinc (mean concentrations 106.7 μg/g and 168.5 μg/g dry weight, respectively; $p = 0.003$).

The results for arsenic in chick down reflect the site differences found in the egg results, with concentrations significantly higher in the samples from the Poole site than those from the Lymington site. These results indicate that arsenic concentrations in feathers may provide a good indication of concentrations in eggs and of local pollution. However, it is important to consider the very small sample size analysed for feathers in this study and the fact that the concentration of arsenic in many of the Lymington feather samples was below the limit of detection. The usefulness of feathers to provide a good indication of arsenic concentrations in concentrations in internal tissues is an area that would benefit from future research.

The limited amount of data in the literature regarding the relationship between zinc concentrations in feathers and internal tissues suggests that feather concentrations may be a poor indicator of tissue concentrations. Zinc is an essential element required for feather formation, and it is suggested that metals that are essential for feather formation may be regulated homeostatically (i.e. the transport and uptake of the metal is controlled in the body by the nervous system) to maintain adequate concentrations for feather production and growth (Clarkson, 1986; Burger, 1993). As concentrations of essential elements such as zinc are carefully regulated in the body, changes in the level of exposure to zinc do not affect all tissues uniformly. The majority of the body burden of zinc is deposited in muscle and bone (Jackson, 1989) and some tissues are therefore considered to be poor indicators of the zinc concentration in the body; feathers are generally thought to fall into this category (see Section 6.1.2). However, one of the ways in which birds regulate the body levels of metals, including zinc, is by sequestration into eggs, and eggs are believed to provide a good indication of tissue levels of zinc. The fact that the difference between sites in zinc feather concentrations is in agreement with the difference found for egg samples suggests that feathers, like eggs, may provide a good

indication of tissue concentrations. This is also supported by literature data for sediment zinc concentrations from previous studies, which have also shown concentrations in the Poole area to be higher than those in sediments from the Lymington area (see Section 5.4.2). Further research into the regulation of zinc in the body and relative tissue contents of zinc in birds is required to clarify the relationship between dietary zinc exposure, tissue concentrations and feather concentrations.

Cobalt concentrations were found to be significantly higher in the down sampled from chicks on the Poole colony than those on the Lymington colony (mean feather concentrations 0.082 μg/g and 0.005 μg/g dry weight, respectively; $p = 0.001$). No previous studies could be found examining the sequestering of cobalt in feathers, or comparison of feather concentrations with concentrations in other tissues. Egg concentration data in this study showed cobalt concentrations to be significantly higher in the post-collection Lymington eggs than the Poole eggs, in contrast to the feather data, indicating that feathers may not be a good indicator of cobalt concentrations as they do not reflect the differences between sites found for cobalt concentrations in eggs. However, it is again important to consider the very small sample size analysed for feathers, and the fact that data obtained in this study were for eggs only; sediment data for cobalt concentrations in the Poole and Lymington sediments from previous studies have shown very little difference between the two sites (Section 5.4.2). As no other tissues were sampled, it is impossible to make inferences as to whether cobalt concentrations in feathers provide a good indication of concentrations in internal tissues, and this is an area that would benefit from future research.

There was no significant difference between the down from chicks nesting on the Lymington and Poole colonies in terms of cadmium concentration, and concentrations in nearly all samples were undetectable. Previous studies have found that cadmium is not sequestered in appreciable amounts to feathers, and correlations between tissue concentrations and feather concentrations are generally reported to be poor (see Section 6.1.1). The fact that concentrations of cadmium in chick down were largely undetectable in the present study supports the findings of previous studies that cadmium is not sequestered into feathers in appreciable amounts.

Although iron concentrations in feathers are usually high (iron being required in large amounts for feather formation), feather concentrations are usually poorly correlated with tissue concentrations and concentrations of iron are thought to be regulated homeostatically (Clarkson, 1986; Burger, 1993). Again, in the present study the concentrations of iron in gull eggs were found to be significantly different between the sites, with significantly greater concentrations of

iron measured in the eggs from the Lymington site (post-collection) compared to those from the Poole site. This difference between sites is not reflected in the data for chick down, with mean iron concentrations slightly higher in down from Lymington chicks than Poole chicks (35.6 µg/g and 22.9 µg/g dry weight, respectively) but no significant difference between sites. These data suggest that feather concentrations of iron may not provide a good reflection of tissue concentrations, as the site differences found in the egg concentration data in this study are not reflected in the feather data. However, again it is important to consider the fact that data obtained in this study were for eggs only, and data for iron concentrations in sediments from the Lymington and Poole areas from previous studies have shown very little difference between the two sites (28132 µg/g and 29290 µg/g for Lymington and Poole, respectively; Bryan & Langston, 1992). As no other bird tissues were sampled or current sediment data obtained, it is not possible to draw a firm conclusion as to whether iron concentrations in feathers provide a good indication of concentrations in internal tissues, and this is an area that would benefit from future research.

No previous studies could be found regarding the relationship between feather concentrations of vanadium with those in tissues. Like zinc, vanadium is an essential element for feather formation and the amount sequestered into feathers is thought to be regulated to maintain adequate concentrations; feathers are thus generally considered to be poor indicators of the vanadium concentration in the body (Clarkson, 1986; Burger, 1993). In the present study, vanadium concentrations were similar in feathers from both the Lymington and Poole chicks (mean concentrations 25.8 µg/g and 29.5 µg/g dry weight, respectively), which reflects the results for egg concentrations. On the other hand, if concentrations of vanadium in feathers are regulated internally, concentrations in feathers would be expected to be similar, regardless of the site and the dietary exposure of the bird to vanadium. Based on the limited data, it is difficult to assess whether feathers can be used as indicators of tissue concentrations of vanadium, and further investigation into the regulation in the body and relative tissue contents of vanadium in birds is required to clarify the relationship between dietary exposure, tissue concentrations and feather concentrations.

Information regarding the sequestering of copper, manganese, nickel and selenium into feathers is conflicting, with some authors reporting correlations between feathers and other tissues (Burger, 1993; Gochfeld *et al.*, 1996), and others reporting no correlation (Dauwe *et al.*, 2000; Dauwe *et al.*, 2004). Copper and manganese are essential elements in feather formation and the concentrations of these metals are thought to be regulated internally to maintain relatively stable concentrations in all tissues, including feathers (Clarkson, 1986; Burger, 1993). In the present

study, concentrations of copper, manganese and selenium were significantly higher in the eggs from the Poole site than those from the Lymington site. Feather data for copper and selenium show concentrations to be similar for chick down from both the Lymington and Poole sites (respective mean concentrations of 3.79 μg/g and 3.61 μg/g dry weight for copper, and 1.76 μg/g and 1.84 μg/g dry weight for selenium). The mean concentration of manganese in feather samples from Poole chicks is higher than that of the feathers from Lymington chicks (2.36 μg/g and 1.24 μg/g dry weight, respectively), and the mean concentration of nickel in feather samples from Lymington chicks is slightly higher than that of the feathers from Poole chicks (0.47 μg/g and 0.30 μg/g dry weight, respectively); however, neither difference is statistically significant. As the differences in manganese and nickel concentrations in feather samples between the sites reflect the differences in the egg samples, it is possible that feathers may provide a good indication of manganese and nickel concentrations in other tissues, such as eggs, and of local pollution. However, differences between sites for manganese or nickel concentrations in feathers are not statistically significant, unlike the differences in the egg data, and further research in to the correlation of feather concentrations of these metals with concentrations in other tissues would be beneficial to further investigate this relationship. The lack of correlation between the patterns observed in the egg and feather concentrations of these metals suggests that feathers may not be good indicators of tissue concentrations for copper and selenium.

Lead concentrations in feathers are generally thought to provide a good indication of concentrations in other tissues, and a number of authors report correlations between concentrations in feathers and tissues, excrement and food samples (Gochfeld *et al.*, 1996; Dauwe *et al.*, 2000; Dauwe *et al.*, 2004). However, other studies have shown little or no correlation between tissue and feather concentrations of lead (Battaglia *et al.*, 2005) and concentrations of lead in feathers may be confounded by external contamination, for example atmospheric deposition. Feather washing can be used to minimise the amount of external contamination of lead and other metals, and chick feathers can also be examined to minimise the amount exogenous contamination that birds have been exposed to. A number of different feather-washing methods have been employed in studies, whilst other studies have not attempted to wash feathers in any way; all of these different approaches to feather analysis make it difficult to compare the results reported in the literature, and no studies have examined the relationship between lead concentrations in chick down and those in tissues; thus, the usefulness of feathers as indicators of the body burden of lead is far from clear. In the present study, down from chicks less than 48 hours old was analysed in an attempt to minimise the external contamination of feathers, and feathers were washed using the method employed most

frequently by authors analysing metal concentrations in feathers (see Section 6.2.1). The concentrations of lead in feathers in this study were similar for each of the sites, in contrast to the data for egg samples, for which lead concentrations were higher in the post-collection Lymington eggs (Lymington Late eggs, i.e. those which were laid after commercial harvesting had ceased and were able to develop and hatch) than the Poole eggs. Based on the results of this study, it seems that feathers may not provide a good indication of egg concentrations of lead and perhaps, as suggested by previous authors that have also found no correlation between lead concentrations in feathers and other samples, some of the reports of relatively high lead concentrations in feathers and correlations with those in tissues may be attributable to external lead contamination. The results from the feather washing trials in this study confirm that external concentrations of lead (and other metals) contribute significantly to the concentrations measured in feathers, with unwashed feathers containing nearly twice the amount of lead as those that have been washed (see Section 6.2.1 and Appendix D).

As previously mentioned, it is important to note that the mass of the feather samples in this study was very low - all samples less than 30 mg fresh weight - due to the fact that down was obtained by cutting a small amount from the bodies of chicks, and this may have limited the results, particularly in the case of those metals for which concentrations in many of the samples were undetectable, for example cadmium, cobalt and nickel. Chick down was sampled in this study as it is considered to be more reflective of metal concentrations obtained from the egg and diet, and metal concentrations in down are likely to be largely from internal metal loads as chicks have had little exposure to the environment (Section 1.3). When sampling down from live chicks only a small sample can be taken, to ensure that the chick is left with an adequate cover of feathers for warmth, and the only other way to sample larger samples of chick down would be by using a destructive method, or by sampling feathers from chicks that have died of natural causes. The problems with the former option are obvious - non-destructive sampling is always preferable, where possible - and the latter option risks biasing the results as chicks that have died naturally may have been unhealthy and have a greater body burden of contaminants than those chicks that survived. The small mass of feathers analysed in this study means that the concentrations of a number of metals were undetectable in some cases, and it is likely that, had larger samples been obtained, some of these metals would have been present at measurable concentrations.

6.4.2 Comparison with literature data

Concentrations of arsenic, cadmium, lead, manganese and selenium measured in chick down samples in this study are all similar or less than those reported in the literature for feathers of young seabirds of various species in previous studies, with the exception of lead for which concentrations in the present study were slightly higher than those reported in the literature. Unfortunately, no data are available for concentrations of cobalt, copper, iron, nickel, vanadium and zinc in feathers of young seabirds from other studies. Data for adult birds are available for cobalt, copper, iron, nickel and zinc, and concentrations reported in previous studies are of similar order, or higher than, the concentrations measured in this study. However, metal concentrations in feathers have been shown to vary with age and feather type (see Section 1.3), and comparison of the results for concentrations in chick down from the present study with results from studies with adult birds examining various feather types is far from ideal.

Although data were not available for concentrations of vanadium in feathers of juvenile seabirds, the concentrations measured in chick down in this study are around 50 times higher than those reported in field studies examining vanadium concentrations in adult seabird feathers (mean concentration 0.57 μg/g dry weight in literature studies compared to 27.6 μg/g in this study). The vanadium concentrations in eggs in the present study were also 80-150 times higher than those reported for seabirds in field studies in the literature, and this similarity between sample types suggests that feathers may provide a good indication of vanadium concentrations in other tissues, such as eggs. Unfortunately, no data are available in the literature relating the concentrations of metals in chick down to concentrations in internal tissues or negative effects on health and survival of birds; thus it is not possible to make an assessment of the potential impacts of elevated concentrations of lead and vanadium in black-headed gull chick down on the birds themselves.

6.5 Summary

This chapter has demonstrated that feathers may provide a good indication of local pollution and concentrations of arsenic and zinc in black-headed gull chick down, with the patterns in the feather data reflecting the egg data and the potential sources of local pollution. Although the patterns in the feather data for manganese and nickel concentrations reflect those differences found in the egg data, the differences between sites in the feather data were not statistically significant, whereas the egg data revealed statistically differences between the sites for

concentrations of these metals. Cadmium concentrations on feathers were largely below the limit of detection, indicating that cadmium may not be sequestered into feathers in appreciable amounts. Feather concentrations of cobalt, copper, iron, lead and selenium did not reflect the patterns observed in the concentrations in eggs. However, the very small sample size analysed for feathers, and the fact that data obtained in this study were for eggs only, means that it is not possible to conclude that feather concentrations of these metals do not provide a good indication of local pollution or concentrations in eggs, and this area would benefit from future research.

CHAPTER 7. GENERAL DISCUSSION

This thesis has examined the concentrations of heavy metals and selenium in populations of black-headed gulls from different colonies in the UK which have different characteristics and are subject to different sources, types and degrees of pollution. The potential sources of heavy metal and selenium pollution around each of the sites have been examined (Chapter 4), the concentrations of heavy metals and selenium in black-headed gull eggs measured and the potential effects of these contaminants on breeding success assessed (Chapter 5). The partitioning of heavy metals and selenium in the egg has also been investigated. Concentrations of heavy metals and selenium in black-headed gull feathers, specifically chick down, have been measured and the usefulness of feathers as a tool for non-destructive monitoring of metal pollution discussed (Chapter 6). Comparisons were also made between a site on which the black-headed gull colony is subject to high-level commercial harvesting of eggs and an unharvested site, and the impacts of commercial egg collecting on reproductive success and contaminant concentrations in eggs investigated (Chapters 2 and 5). Differences in the nesting density of black-headed gulls between the collected and uncollected sites have been explored, and the effects of nesting density on egg size assessed (Chapter 3).

Heavy metals in air, soil, and water are a global problem and present a growing threat to the environment. Even at concentrations insufficient to cause death or other acute effects, these metals may have profound consequences for birds, causing increased susceptibility to disease or other stresses, changes to normal behaviour patterns and decreased reproductive success (Heinz, 1974; Scheuhammer, 1987; Burger & Gochfeld, 1995b; Heinz *et al.*, 1999; see also Section 1.1). As well as exhibiting direct effects, heavy metal pollution may also affect bird populations through effects on the abundance of prey organisms (Bryan & Langston, 1992). Seabirds feeding at upper trophic levels are exposed to relatively high concentrations of contaminants in their prey, making them particularly vulnerable to pollution and susceptible to the effects of bioaccumulation, and thus able to provide information on the extent of contamination in the whole food chain. In addition, sampling during the breeding season provides a reflection of local pollution, as breeding gulls obtain all food locally prior to and during breeding (Gorke & Brandl, 1986).

Black-headed gulls are on the 'Amber' list of the British Trust for Ornithology (BTO) Birds of Conservation Concern 2002-2007, indicating that they are of 'medium' conservation concern (BTO, 2007). The licensed commercial collection of their eggs for culinary purposes means that

black-headed gulls face a unique pressure amongst UK seabirds and enables interesting comparisons between collected and uncollected colonies, in addition to comparisons between breeding sites in different parts of the UK with differing climates and influenced by different types and levels of pollution. Comparisons have been made between colonies on the south coast of England, one subject to commercial collection (Lymington) and one uncollected (Poole) and a smaller colony in the northeast of England that is subject to very low-level non-commercial collection (Raby).

This study has been the first to report on a number of aspects of heavy metal pollution and avian reproduction, including:

- the effect of commercial egg harvesting on the physical characteristics of the egg, comparing pre- and post-collection eggs from the same site;
- the relationship between nesting density and egg size and dimensions on a typical black-headed gull colony;
- the effect of commercial egg harvesting on nesting density;
- the impacts of concentrations of heavy metals and selenium on the intrinsic quality of the egg, as reflected by yolk:albumen ratio, and the effects of cobalt, copper, iron, manganese, nickel, selenium and vanadium on eggshell thickness and shell index;
- the partitioning of heavy metals between the egg contents (yolk and albumen);
- the effect of commercial egg harvesting on metal concentrations in eggs; and
- concentrations of heavy metals and selenium in the down feathers of young chicks.

The following discussion will bring together and evaluate some of the key findings from the data chapters of this study, point out some likely implications of these findings and suggest areas of study that would benefit from future research.

7.1 Heavy metals in black-headed gull eggs and feathers

This study has provided general information regarding the level of heavy metal and selenium exposure of black-headed gulls on three different UK colonies, and identified some potential sources of metal contamination in these areas. The concentrations of arsenic, copper, nickel,

selenium and vanadium measured in black-headed gull eggs in this study were consistently high relative to those reported in previous field studies with other seabird species, for all sites examined. The elevated concentrations of these metals in black-headed gull eggs compared with those of other gull and seabird species may be associated with the considerable amount of time spent foraging on land, in comparison. Black-headed gulls may therefore be exposed to more land-based sources of metals than most other species of seabird. Arsenic, copper, nickel, selenium and vanadium are all metals associated with urban and agricultural runoff (see Section 4.2), and thus birds feeding on farmland and in urban areas might be exposed to more elevated levels of these pollutants than seabirds that spend little time on land. Mean concentrations were particularly high at all sites compared to those reported in the literature for vanadium, with concentrations in this study over 80 times higher than the concentrations reported in the eggs of other seabird species. The concentrations of vanadium in black-headed gull eggs in this study were consistently high, regardless of the site and the potential sources of pollution. In addition, vanadium concentrations in down from chicks from the Poole and Lymington colonies were also considerably higher than those reported in the literature for feathers of other seabirds. As the concentrations of vanadium were consistently high for all the sites sampled in this study, it seems that the concentrations of vanadium measured in the eggs and feathers of black-headed gulls in this study might be considered representative of the species. As previously mentioned, black-headed gulls are exposed to more land-based sources of contaminants than most other seabird species; increased exposure to vanadium may result from foraging in urban areas and on farmland where elevated levels of vanadium may be encountered owing to its release from vehicle emissions and use in mineral fertilisers (Denton *et al.*, 1997; Legret & Pagotto, 1999). No previous studies have examined the concentrations of vanadium in the eggs or feathers of black-headed gulls, and further research would be required to assess whether the vanadium concentrations measured in the present study can be considered 'normal' for this species.

The analysis of heavy metals and selenium in this study has highlighted the importance of considering diffuse and historical pollution in addition to current industrial discharges, with the Poole samples having higher concentrations of the majority of metals than the Lymington samples, in spite of the larger scale of industrial and other point-source discharges around the Lymington site. Diffuse pollution is defined as pollution arising from many sources, which may be small individually, but the collective impact of which can be damaging. This confirms the general opinion in the scientific community that, with point source discharges carefully monitored and subject to tight consents, diffuse pollution, which by its very nature is extremely difficult to quantify and control, has become increasingly important in terms of environmental

pollution (CIWEM, 2004). As heavy metals do not break down in the environment, historical pollution is also very important. Poole Harbour, particularly the Holes Bay area, has been historically subject to heavy pollution as a result of a number of industrial releases of heavy metals, most notably discharges from a large chemical manufacturing plant, that have left a legacy of heavy metal pollution in the sediments of the Harbour. The impact of historical pollution as a result of anthropogenic activities is also reflected in the egg samples taken from the gulls nesting on the Raby estate in this study. The area of the Raby site was historically mined for lead, with the gull colony itself being located on old lead mining dams. In this study, concentrations of lead in eggs from the Raby colony were significantly higher than eggs from any other colony and approximately six times higher than those reported in literature for eggs of other gull species, reflecting the elevated levels of lead in the area.

Very few studies have examined the effects of metals on the physical characteristics of the egg. Some authors have examined the impacts of certain metals on eggshell thickness, namely for arsenic, cadmium, lead, vanadium and zinc (Haegele & Tucker, 1974; Leach *et al.*, 1979; Ousterhout & Berg, 1981; Hussein *et al.*, 1988; Davis *et al.*, 1995; Bressman *et al.*, 2002); there is no information regarding the impacts of cobalt, copper, iron, manganese, nickel, or selenium on eggshell thickness, and this study is the first to examine the effects of any of these metals on other egg characteristics, such as the relative amounts of yolk and albumen. Concentrations of cobalt, iron and nickel in black-headed gull eggs in this study were significantly correlated with the wet weight of albumen and the yolk:albumen ratio (Section 5.4.1), with the wet weight of albumen increasing and the yolk:albumen ratio decreasing with increasing metal concentration. Concentrations of cobalt, iron and nickel were also found to increase with relaying, with concentrations in post-collection eggs significantly higher than pre-collection eggs from the same site (see also Section 7.2).

Concentrations of arsenic, cadmium, copper, lead, manganese, selenium, vanadium and zinc measured in this study do not appear to have any significant effect on the physical characteristics of the egg in terms of shell thickness, shell index, mass of yolk, albumen, shell or total egg, egg volume, egg length and breadth or yolk:albumen ratio (Section 5.4.1). Although the concentrations of these metals in this study have no significant effect on the physical characteristics of the egg, even for those which were measured at concentrations far higher than those in gull and seabird eggs in previous studies, it cannot be conclusively said that the metals examined in this study have no significant effect on the breeding success of black-headed gulls, as no investigation was made into the hatching success, fledging or survival of the chicks on the sites. Further study examining the hatching and post-hatching success of chicks on sites subject

to high levels of metal pollution compared with less polluted sites would go a step further towards clarifying the relationship between metals concentrations and breeding success of black-headed gulls (see Section 7.3).

Although some authors have examined metal partitioning between eggshell and contents, only one previous study has examined partitioning between the egg contents themselves, and only for selenium (Magat & Sell, 1979). This thesis has addressed some of the gaps in the knowledge regarding the partitioning of heavy metals and selenium in eggs, providing information on the partitioning of metals not only between egg contents and shell, but also between yolk and albumen (Section 5.3.3). The results have shown that, in black-headed gull eggs, arsenic, iron, manganese, lead, selenium, vanadium and zinc are partitioned more to egg contents than shell, and cadmium, cobalt and nickel are found at higher concentrations in the shell than egg contents. Copper is partitioned roughly equally between contents and shell. In terms of partitioning within the egg contents, the results of this study show that arsenic, copper, vanadium and selenium concentrations are higher in the albumen than the yolk, and cobalt, iron, manganese, nickel, lead and zinc concentrations are higher in the yolk than the albumen. For cadmium, which is partitioned mainly to eggshell, the concentrations in egg contents were largely undetectable, and thus partitioning in contents is insignificant. The information provided in this study with regard to partitioning of heavy metals and selenium in the egg will allow future studies to adopt a more targeted approach to the analysis of metals in eggs. Previous studies have often focused on egg contents and have neglected to analyse eggshell at all (for example: Hernández *et al.*, 1988; González & Hiraldo, 1988; Burger & Gochfeld, 1991; Baranowska *et al.*, 2005), and analysis of egg contents has examined homogenised contents rather than separate components. For some metals, namely cadmium, cobalt and nickel, previous studies that have reported low or undetectable concentrations in eggs (for example: Sell, 1975; Scheuhammer, 1987; Burger & Gochfeld, 1993; Braune & Simon, 2004) may have done so because the authors neglected to analyse the egg shell. In addition, it may be prudent for future studies examining metals in egg contents to focus the analysis on one particular component of the egg; for example, in terms of egg contents, iron, lead, manganese and zinc are present almost entirely in the yolk and, when looking at low concentrations, analysis of homogenised egg contents will only serve to dilute the concentrations of these metals in the sample. For these metals, particularly when concentrations are very low, it may be argued that analysis of yolk rather than egg contents as a whole would provide the best assessment of concentrations on the egg contents. Ideally, in order to obtain accurate results for total concentrations in the egg, all three egg components should be examined separately.

A number of authors have measured concentrations of heavy metals and selenium in seabird feathers. However, studies have shown that metal concentrations in feathers vary greatly according to the type of feather sampled and the age and sex of the bird, and exogenous contamination means that metal concentrations measured in feathers in many studies may not provide an accurate reflection of the body burden. This study has demonstrated that washing feathers removes at least some of this external contamination and, combined with the sampling of down of very young chicks (less than 48-hours old), the level of external contamination was minimised. Although some studies have examined metal concentrations in the feathers of juvenile birds, this study is the first to examine metal concentrations in chick down. In addition, very few studies have investigated the relationship between feather concentrations and those in other tissues for metals other than mercury, lead and cadmium. This study has compared the trends observed in metal concentrations in eggs between sites with those observed for feathers, in order to provide some indication of the usefulness of feathers as indicators of heavy metal and selenium contamination. The results suggest that feathers may be good indicators of arsenic and zinc contamination, with the significant site differences observed in the egg data reflected in the feather data. The patterns in the egg data were also reflected in the feather data for manganese and nickel, indicating that feathers may also provide a reflection of concentrations in eggs and of local pollution for these metals. However, the differences between sites for the feather data for these metals were not statistically different, while the differences in the egg data were significant. In line with the egg data, concentrations of vanadium in the feather samples were similar for each of the sites. However, vanadium concentrations in eggs were considerably higher than those reported in previous studies with seabirds, and concentrations measured in feathers were also considerably higher than those measured in previous studies; thus it is possible that sequestering of vanadium into feathers may be significant, and feathers may provide a good indication of vanadium contamination. The differences observed between sites in the egg data are not reflected by the feather data for cobalt, copper, iron, lead and selenium, suggesting that feathers may not provide a good indication of contamination for these metals. However, the nature of the sampling technique used in this study meant that the down samples taken were very small and concentrations measured were very low and, in some cases, largely undetectable. The small sample mass and low metal concentrations in the samples have unfortunately limited the conclusions that can be drawn from the feather data reported in this study, and it would be beneficial to analyse larger samples to make a more accurate assessment of metal concentrations in chick down (see Section 7.3).

7.2 Impacts of commercial egg harvesting

Black-headed gull eggs have been harvested on the Lymington colonies for centuries, a practice which has been licensed since the implementation of the Wildlife and Countryside Act (1981). Data regarding the effects of commercial harvesting on reproductive success are limited, and no previous studies have examined the effects of commercial egg collecting on the concentrations of metals in eggs, nor the impacts on nesting density. In addition to the concentration of metals in eggs as a result of relaying forced by commercial egg collection, this study is also the first to analyse differences between pre- and post-collection eggs from the same commercially harvested colony in terms of the physical characteristics.

The results from the investigations into the differences in size, dimensions and composition of black headed gull eggs have shown the post-collection Lymington eggs to be of a lower quality than the first-laid, pre-collection eggs and the eggs from the uncollected Poole site, as indicated by the decreased yolk:albumen ratio. Although no difference in egg size and dimensions (length and breadth) was observed between pre- and post-collection eggs, the post-collection eggs contained a significantly greater mass of albumen and a significantly lower ratio of yolk:albumen. The ratio of yolk to albumen is generally considered to provide a better reflection of the intrinsic quality of the egg than egg size, as larger eggs often contain relatively more albumen and less yolk (Romanoff & Romanoff, 1949; Parsons, 1976a; Nisbet, 1978; Ricklefs *et al.*, 1978; Finkler *et al.*, 1998; Lessells *et al.*, 2002) and, as yolk is the food reserve for the developing chick, large-yolked eggs provide more lipid energy and hatch larger chicks (Carey, 1996; Finkler *et al.*, 1998). In the one previous study examining the effects of commercial egg harvesting on black-headed gull eggs, the eggs from collected colonies had significantly thinner shells than those from uncollected colonies (Wood *et al.*, 2009). In the present study no significant difference was found for eggshell thickness or shell index between eggs from the collected Lymington colony and the uncollected Poole colony. Eggshell thickness and shell index were higher in the Lymington eggs sampled prior to commercial collection than those sampled at the end of the collection period; however, neither difference was statistically significant. Eggs with thinner shells are more likely to break and thinner shells may lead to increased water loss, possibly leading to desiccation of the egg and embryo and thus resulting in decreased hatching success (Davis & Ackerman, 1987; Eeva & Lehikoinen, 1995; Nybø *et al.*, 1997). Indeed, in a previous study Wood *et al*. (2009) report a significantly higher proportion of desiccated and un-hatched eggs on the collected Lymington colony compared with the uncollected Poole colony.

One of the principal difficulties with gauging the impact of egg harvesting is to find sufficient replicate sites to avoid confounding influences such as colony size and nest position, and the reduced geographical range of black-headed gulls means that insufficient colonies exist within the same geographical area to estimate interactions between all potential effects (Wood, 2007). In order to minimise variation, this study has compared collected and uncollected colonies of similar size, both off the south coast of England, and nests from similar positions within the colony. To validate this strategy, the effect of nest position and nesting density on egg size and dimensions was examined. The relationship of nest position and nesting density with breeding success of black-headed gulls has only been examined in one previous study, which was carried out nearly 50 years ago and examined an atypical colony nesting on sand dunes and, being far more accessible to land predators than the more typical 'island' colonies, with an exceptionally high mortality rate (Patterson, 1965). This study is the first to examine the relationship between nest density and breeding success on a typical black-headed gull colony. The results show that, within the main colony, there was no variation in egg size with nest location or with nesting density, which is in agreement with the findings of the Patterson (1965) study. However, this study examined only egg size and dimensions, and future studies examining hatching and survival of chicks would be beneficial to further examine the relationship between nest location, nesting density and breeding success (see Section 7.3).

The average nesting density on the Lymington colony was found to be significantly lower than on the Poole colony (Chapter 3), which could be an indication that nesting on the Lymington colony is less desirable than nesting on the Poole colony, owing to the fact that the gulls nesting on the Lymington colony are forced to relay several times as a result of the egg collecting. In addition, the Lymington islands are far more prone to flooding (Wood, 2007) than the islands located in the much more sheltered Poole Harbour; this flooding leads to loss of eggs and young chicks, which may make the Lymington salt marshes less desirable habitat that the Poole salt marshes. Loss of habitat due to the rapid erosion of the salt marsh islands that the gulls nest on may also be a contributing factor; both the Poole and Lymington marshes are eroding, however, the salt marsh at Lymington is eroding at twice the rate of the salt marsh at Poole, with the seaward edge of the salt marsh eroding at a rate of around three metres per year (SCOPAC, 2004a; SCOPAC, 2004b) and 81% of the marsh lost between 1921 and the early 2000s as a result of this erosion (New Forest District Council, 2004a).

This study is the first to examine the concentration of metals in the egg as a result of forced relaying due to commercial egg harvesting. The results have demonstrated the concentration of metals through the laying sequence, with the first-laid eggs of the birds on the collected

Lymington colony containing lower concentrations of a number of metals than those eggs laid at the end of the collection period (Chapter 5). As a result of harvesting, the gulls will have laid several clutches prior to this clutch of 'late' eggs, and these eggs (which are those that would finally be left undisturbed and allowed to develop and hatch) have been shown by this study to contain significantly higher concentrations of cobalt, iron and nickel than the first laid eggs, and a significantly lower yolk:albumen ratio. Thus, continued relaying as a result of loss of eggs through commercial egg harvesting has been shown to lead to concentration of the heavy metals cobalt, iron and nickel, which in turn are associated with a decrease in egg quality, as indicated by the relative amount of yolk.

Although the average concentrations of arsenic, lead, manganese and selenium were higher in post-collection eggs compared to pre-collection eggs, the differences were not statistically significant, and the concentrations of metals other than cobalt, iron and nickel in the post-collection eggs were not associated with any effect on the physical characteristics of the egg (Section 5.4.1), and are not of a level thought to have a negative impact on breeding success (Section 5.4.3). However, the increased concentration of these metals in eggs as a result of commercial egg harvesting could have consequences for black-headed gulls nesting in areas with higher levels of metal pollution, or following a pollution incident, as the concentration of metals in the egg could then result in levels in the egg sufficient to have a negative impact on the egg or the developing embryo.

7.3 Future work

This thesis has examined a number of aspects of heavy metal and selenium pollution and avian reproduction that have not previously been investigated. The results presented have also generated a number of interesting ideas and questions which provide a strong foundation for future work, and highlighted some areas where information is sorely lacking.

It is clear that toxicity studies with heavy metals and selenium, particularly chronic dietary studies (i.e. long term, repeated exposure), examining the amount of metals sequestered into the eggs of breeding female birds as a result of dietary exposure, and impacts of these concentrations on reproductive success such as egg quality, hatching success, fledging success, and survival, are required to increase understanding into the ecotoxicological effects of metals. Studies regarding the effects of metals on reproductive success are extremely limited, particularly for metals other than cadmium, lead and selenium, and most have been carried out

only with domestic bird species. Although the present study has shown that the concentrations of metals measured at the sites examined have not had a significant impact on the eggs of black-headed gulls, the hatching success and chick fledging and survival have not been examined, and thus no definite conclusion can be drawn regarding the effects of heavy metals and selenium on the breeding success of the birds. Further study examining the hatching and post-hatching success of chick on sites subject to high levels of metal pollution compared with less polluted sites would go a step further towards clarifying the relationship between metals concentrations and breeding success of black-headed gulls. As the egg must be destroyed in order to perform metal analysis, the metal concentrations in the egg and the resultant chick cannot possibly be examined. However, it may be possible to examine metal concentrations in one egg of the clutch and then monitor the hatching and fledging success of the chicks hatching in the same nest, and relate these to metal concentrations in the sampled egg.

Given the importance of speciation in terms of the toxicity of some metals (for example arsenic, lead, selenium and vanadium), studies examining the speciation of metals in the egg would be beneficial, and may also provide further insight into the sources of the metals.

For some metals, particularly iron, nickel and vanadium, further field studies examining the concentrations in seabird eggs would be extremely valuable with regard to making comparisons between different species and providing an indication of general concentrations of these metals in eggs. It would also be beneficial to examine the metal concentrations in the environment (water, sediments and so on) and in prey organisms at each of the sites, and relate these to the concentrations of metals measured in black-headed gull eggs and feathers. This would further clarify the relationship between exposure of the gulls to heavy metals and how this is reflected in egg and feather samples, and would also provide information as to whether concentrations of metals are of an order likely to have a detrimental effect on prey organisms such as shellfish, worms, insects and fish, resulting in a prey shortage and potentially having a negative effect on the gulls and other bird species in the area. Examination of failed eggs and chicks found dead through no obvious cause would also be useful to assess whether these chicks have abnormally high body burdens of metals. The human health effects of consuming wild birds eggs, which may contain elevated levels of contaminants, is also an area of research worthy of investigation.

The results from this study have shown that nesting density does not have a significant effect on egg size. Again, these results do not provide a conclusive answer to the question of whether nest density has an effect on reproductive success, and further study examining hatching success, fledging and survival of chicks would provide a more accurate indication of the

relationship between breeding success and nest density. This area of research would also benefit from extension of the range of nests examined, to include those birds nesting outside the main colony as well as those in the centre and at the edges of the main colony.

Data regarding metal concentrations in seabird feathers are limited or non-existent, particularly for juvenile birds. Further study regarding the concentrations of heavy metals and selenium in seabird feathers would be useful for future research, and future studies concentrating on chick down and using appropriate feather washing techniques, therefore eliminating much of the exogenous contamination and allowing comparison between studies, would be particularly valuable. In addition there is very little information in the literature regarding concentrations of metals in feathers compared to those in tissues, and the limited data are often conflicting. Further research into the regulation of essential metals in the body and relative tissue concentrations of heavy metals and selenium in birds is required to clarify the relationship between dietary exposure, tissue concentrations and feather concentrations. Dietary studies with young chicks exposed to heavy metals and selenium and the resulting concentrations in down feathers would be particularly useful in order to clarify the relationship between dietary exposure and sequestration into growing feathers.

One of the key problems with the feather analysis in this study was the amount of sample that was able to be collected. The advantage of using chick down for metal analysis in feathers is that, combined with an appropriate washing technique, contamination from external sources can be minimised and the metals in the feathers can be considered to represent the chick's body load of contaminants. However, the sampling of down from live chicks in this study meant that the mass of feathers that could be sampled was limited, as it would have been detrimental to the chicks to take a large amount of down and leave them exposed to cold conditions and with little protection against the elements. Unfortunately, the small amount of feathers analysed in this study meant that a number of metals were undetectable in the vast majority of the samples, and it is likely that concentrations of these metals, namely arsenic, cadmium, cobalt and nickel, may have been detectable under different circumstances. Although this study is the first to examine the concentrations of heavy metals and selenium in chick down, the small sample mass and low metal concentrations therein have limited the conclusions that can be drawn from the data reported here. It would be beneficial to analyse larger samples or use a more sensitive analytical method to make a more accurate assessment of the concentrations in chick down and to better examine the usefulness of feathers as indicators of heavy metals and selenium concentrations in the body. One way of overcoming this issue would be to use a high-resolution ICP-MS (HR ICP-MS), which has a higher sensitivity than standard ICP-MS and is capable of

measuring concentrations of trace elements at parts-per-trillion (ppt), or even parts-per-quadrillion (ppq) level (EAI, 2010). Larger down samples could also be obtained, either by pooling small samples taken from a number of chicks, by examining down and tissues of dead chicks, or by destructive sampling. The latter two options allow for direct comparison to be made between heavy metal and selenium concentrations in the tissues and those in the down, which would provide much-needed clarification regarding the sequestering of metals into feathers. However, the risk with sampling feathers and tissues from chicks that have died of natural causes is that those chicks could have an unrepresentative body burden of contaminants as they may have been produced by birds that have been particularly affected by pollutants (Furness *et al.*, 1993); thus sampling of tissues from dead chicks may not provide a true reflection of metal levels in the colony as a whole. Destructive sampling, although not always desirable, would be the most accurate method of assessing the metal levels in the chicks of the colony as a whole, and relating the concentrations in chick down to concentrations in body tissues. If chicks found dead were sampled alongside healthy chicks, this would also enable comparison between the metals in tissues of apparently healthy chicks and those that died as a result of unknown causes.

7.4 Concluding remarks

This study has provided information regarding the concentrations of heavy metals and selenium in black-headed gull eggs and feathers, and has demonstrated the ability of metal concentrations in eggs to provide a reflection of local pollution. The partitioning of metals in eggs has been investigated, and the findings will enable future studies to adopt a more targeted approach to metal analysis in eggs; this is particularly important as the concentrations of many heavy metals in eggs are very low. The usefulness of feathers in providing an indication of local pollution and concentrations in eggs has been demonstrated for cadmium and zinc. However, there were a number of limitations to this study, particularly in terms of the mass of feather samples analysed, and further investigation into the potential of feathers to provide an indication of local pollution and metal concentrations in other tissues would be a useful and interesting topic for future studies.

Nesting density and nest location (centre of colony vs colony edge) has been demonstrated to have no significant effect on the size and dimensions of black-headed gull eggs. However, only one measure of reproductive success was investigated (egg size), and investigations into the relationship between nesting density, nest location and reproductive success in black-headed

gulls would benefit from examining other measures of breeding success, beyond the size of the egg.

The licensed collection of black-headed gull eggs on the South coast of England has been carried out for centuries and, although the gulls will replace lost eggs, the production of eggs demands high energy and requires mobilisation of body reserves. This study has shown that the licensed collection of black-headed gull eggs is having a negative impact on egg quality (in terms of yolk:albumen ratio and possibly shell thickness and shell index) and leads to an increase in the concentration of some metals, particularly cobalt, iron and nickel, in the egg as a result of repeated relaying. The increased concentrations of cobalt, iron and nickel in black-headed gull eggs after gulls have been forced to relay several times following commercial egg harvesting are significantly correlated with a decrease in the intrinsic quality of the eggs, indicated by yolk:albumen ratio. In light of these results, the level of commercial egg collecting carried out on black-headed gull colonies may need to be reviewed, especially considering their conservation status. This would particularly apply to breeding colonies located on coastal islands, as these gulls are also threatened by destruction of habitat associated with sea level rise, climate change and salt marsh erosion and dieback (Colenutt, 2005; Wood, 2007; Williams *et al.*, 2009; Williams, *in preparation*).

REFERENCES

Abdel-Mageed, A. B. & Oehme, F. W. 1990. A review of the biochemical roles, toxicity and interactions of zinc, copper and iron: III. Iron. *Veterinary and Human Toxicology*, **32,** 324-328.

ABP. 2007. Port of Southampton. Associated British Ports (ABP) Holdings Ltd.: http://www.abports.co.uk/custinfo/ports/soton.htm.

Agusa, T., Matsumoto, T., Ikemoto, T., Anan, Y., Kubota, R., Yasunaga, G., Kunito, T., Tanabe, S., Ogi, H. & Shibata, Y. 2005. Body distribution of trace elements in black-tailed gulls from Rishiri Island, Japan: age-dependent accumulation and transfer to feathers and eggs. *Environmental Toxicology and Chemistry*, **24,** 2107-2120.

Ahmad, S. N. 1977. The geochemical distribution and source of copper in the metalliferous mining region of Southwest England *Mineralium Deposita* **12,** 1-21.

Ainley, D. G. & Lewis, T. J. 1974. The history of Farallon Island marine bird populations. *Condor*, **76,** 432-446.

Al-Swaidan, H. M. 1996. The determination of lead, nickel and vanadium in Saudi Arabian crude oil by sequential injection analysis/inductively-coupled plasma mass spectrometry. *Talanta*, **43,** 1313-1319.

Albrecht, A. 2003. Validating riverine transport and speciation models using nuclear reactor-derived radiocobalt. *Journal of Environmental Radioactivity*, **66,** 295-307.

Alemi, M. H., Goldhamer, D. A., Grismer, M. E. & Nielsen, D. R. 1988. Elution of selenium from contaminated evaporation pond sediments. *Journal of Environmental Quality*, **17,** 613-618.

Alloway, B. J. 1995. *Heavy Metals in Soils, Second Edition*. London: Blackie Academic and Professional.

Altmeyer, M., Dittman, J., Dmowski, K., Wagner, G. & Müller, P. 1991. Distribution of elements in flight feathers of a white-tailed eagle. *Science of the Total Environment*, **105,** 157-164.

Ambrose, R. E., Matz, A., Swen, T. & Bente, P. 2000. Environmental contaminants in American and Arctic peregrine falcon eggs in Alaska, 1979-95. In: *Technical Report NAES-TR-00-02*, pp. 67: Ecological Services Fairbanks, US Fish and Wildlife Service.

Amundsen, T., Lorentsen, S. & Tveraa, T. 1996. Effects of egg size and parental quality on early nestling growth: an experiment with Antarctic petrel. *Journal of Animal Ecology*, **65,** 545-555.

Anderlini, V. C., Connors, P. G., Risebrough, R. W. & Martin, J. H. 1972. Concentrations of heavy metals in some Antarctic and North American sea birds. In: *Proc. Colloq. Conservation Problems in Antarctica* (Ed. by Parker, B. C.), pp. 49. Lawrence, Kansas: Allen Press.

Anderson, D. W. & Hickey, J. J. 1976. Dynamics of storage of organopollutants in herring gulls. *Environmental Pollution*, **10,** 183-200.

Antolos, M., Roby, D. D., Lyons, D. E., Anderson, S. K. & Collis, K. 2006. Effects of nest density, location, and timing on breeding success of Caspian Terns. *Waterbirds*, **29,** 465-472.

Appelquist, H., Asbirk, S. & Drabaek, I. 1984. Mercury monitoring: mercury stability in bird feathers. *Marine Pollution Bulletin*, **15,** 22-24.

Armitage, P. D. 2006. Long-term faunal changes in a regulated and unregulated stream - Cow Green thirty years on. *River Research and Applications*, **22,** 947-966.

Arnold, R. L., Olson, O. E. & Carlson, C. W. 1973. Dietary selenium and arsenic additions and their effects on tissue and egg selenium. *Poultry Science*, **52,** 847-854.

Aspinall, S. J. & Tasker, M. L. 1992. *Birds of the Solent*. Aberdeen: JNCC

Aspinall, S. J., Taverner, J. H. & Wiseman, E. J. 1993. History of Black-headed gull colonies in Hampshire and neighbouring counties. *British Birds*, **86,** 103-114.

ATSDR. 1990. Toxicological profile for copper. In: *Agency for Toxic Substances and Disease Registry*. Atlanta, Georgia: US Department of Health and Human Services.

ATSDR. 2004. Toxicological profile for vanadium. In: *Agency for Toxic Substances and Disease Registry*. Atlanta, Georgia: US Department of Health and Human Services.

ATSDR. 2007. Toxicological profile for lead. In: *Agency for Toxic Substances and Disease Registry*. Atlanta, Georgia: US Department of Health and Human Services.

Baranowska, I., Barchanska, H. & Pyrsz, A. 2005. Distribution of pesticides and heavy metals in trophic chain. *Chemosphere*, **60,** 1590-1599.

Bargagli, R. 2000. Trace metals in Antarctica related to climate changes and increasing human impact. *Reviews of Environmental Contamination and Toxicology*, **166,** 129-173.

Bargar, T. A., Scott, G. I. & Cobb, G. P. 2001. Maternal transfer of contaminants: case study of the excretion of three polychlorinated biphenyl congeners and technical-grade endosulfan into eggs by white leghorn chickens (*Gallus domesticus*). *Environmental Toxicology and Chemistry*, **20,** 61-67.

Barnes, G. 2009. Landforms and flora in Southern Hampshire, England. http://geologyecology.suite101.com/article.cfm/new_forest_geology_geomorphology_and_ecology.

Barrett, R. T., Skaare, J. U., Norheim, G., Vader, W. & Froslie, A. 1985. Persistent organochlorines and mercury in eggs of Norwegian seabirds 1983. *Environmental Pollution Series A*, **39,** 79-93.

Battaglia, A., Ghidini, S., Campanini, G. & Spaggiari, R. 2005. Heavy metal contamination in little owl (*Athene noctua*) and common buzzard (*Buteo buteo*) from northern Italy. *Ecotoxicology and Environmental Safety*, **60,** 61-66.

Becker, P. H. 1989. Seabirds as monitor organisms of contaminants along the German North Sea coast. *Helgolander Meeresuntersuchungen*, **43,** 395-405.

Becker, P. H. 1992. Egg mercury levels decline with the laying sequence in Charadriiformes. *Bulletin of Environmental Contamination and Toxicology*, **48,** 762-767.

Becker, P. H. 1995. Effects of coloniality on gull predation on common Tern (*Sterna hirundo*) chicks. *Colonial Waterbirds*, **18,** 11-22.

Becker, P. H. 2003. Biomonitoring with birds. In: *Bioindicators & Biomonitors: principles, concepts and applications* (Ed. by Markert, B. A., Breure, A. M. & Zechmeister, H. G.). London: Pergamon Press.

Becker, P. H., Conrad, B. & Sperveslage, H. 1989. Organochlorines and heavy metals in female herring gulls and in their clutches of known laying sequence. *Vogelwarte*, **35,** 1-10.

Becker, P. H. & Erdelen, M. 1986. Egg size in Herring Gulls (*Larus argentatus*) on Mellum Island, North Sea, West Germany: the influence of nest vegetation, nest density, and colony development. *Colonial Waterbirds*, **9,** 68-80.

Bellrose, F. C. 1951. Effects of ingested lead shot upon waterfowl populations. *Transactions of the North American Wildlife Conference*, **16,** 125-135.

Bennett, C. D. 1992. The influence of shell thickness on hatchability in commercial broiler breeder flocks. *Journal of Applied Poultry Research*, **1,** 61-65.

Bernhard, M. & Andreae, M. O. 1984. Transport of trace metals in marine food chains. In: *Changing metal cycles and human health* (Ed. by Nriagu, J. O.), pp. 143-167. Berlin: Dahlen Konferenzen.

Best, L. B. & Stauffer, D. E. 1980. Factors affecting nesting success in riparian bird communities. *Condor*, **82,** 149-158.

Bird, P., Comber, S. D. W., Gardner, M. J. & Ravenscroft, J. E. 1996. Zinc inputs to coastal waters from sacrificial anodes. *Science of the Total Environment*, **181,** 257-264.

Birge, W. J. & Roberts, O. W. 1976. Toxicity of metals to chick embryos. *Bulletin of Environmental Contamination and Toxicology*, **16,** 319-324.

Birkhead, T. R. 1977. The effect of habitat and density on breeding success in the common guillemot (*Uria aalge*). *Journal of Animal Ecology*, **46,** 751-764.

Birkhead, T. R. & Nettleship, D. N. 1982. The adaptive significance of egg size and laying date in Thick-Billed Murres *Uria Lomvia*. *Ecology*, **63,** 300-306.

Bjorlykke, A. & Thorpe, R. I. 1982. The source of lead in the Osen sandstone lead deposit on the Baltic Shield, Norway *Economic Geology*, **77,** 430-440.

Blom, J. & Lilja, C. 2004. A comparative study of growth, skeletal development and eggshell composition in some species of birds. *Journal of Zoology*, **262,** 361-369.

Blomqvist, D., Johansson, O. C. & Gotmark, F. 1997. Parental quality and egg size affect chick survival in a precocial bird, the lapwing, *Vanellus vanellus*. *Oecologia*, **110,** 18-24.

Blus, L. J., Lamont, T. G. & Neely, B. S. 1979. Effects of organochlorine residues on eggshell thickness, reproduction, and population status of Brown Pelicans (*Pelecanus occidentalis*) in South Carolina and Florida, 1976-79. *Pesticide Monitoring Journal*, **12,** 172-184.

Blus, L. J., Neely, B. S., Jr., Lamont, T. G. & Mulhern, B. 1977. Residues of organochlorines and heavy metals in tissues and eggs of brown pelicans 1967-73. *Pesticide Monitoring Journal*, **11,** 40-53.

Bodek, I., Lyman, W. J., Reehl, W. F. & Rosenblatt, D. H. 1988. Environmental Inorganic Chemistry: Properties, Processes, and Estimation Methods New York: Pergamon Press.

Boersma, P. D., Rebstock, G. A. & Stokes, D. L. 2004. Why penguin eggshells are thick. *The Auk*, **121,** 148-155.

Bogan, J. A. & Newton, I. 1977. Redistribution of DDE in sparrowhawks during starvation. *Bulletin of Environmental Contamination and Toxicology*, **18,** 317-321.

Bolton, M. 1991. Determinants of chick survival in the lesser black-backed gull: relative contributions of egg size and parental quality. *Journal of Animal Ecology*, **60,** 949-960.

Bolton, M., Houston, D. C. & Monaghan, P. 1992. Nutritional constraints on egg formation in the lesser black-backed gull: an experimental study. *Journal of Animal Ecology*, **61,** 521-232.

Bolton, M., Monaghan, P. & Houston, D. C. 1993. Proximate determination of clutch size in the lesser black-backed gull: the role of food supply and body condition. *Canadian Journal of Zoology*, **71,** 273-279.

Booth, D. T. & Seymour, R. S. 1987. Effect of eggshell thinning on water vapor conductance of Malleefowl eggs. *The Condor*, **89,** 453-459.

Bosch, M. & Sol, D. 1998. Habitat selection and breeding success in yellow-legged gulls *Larus cachinnans*. *Ibis*, **140,** 415-421.

Boxall, A. B. A., Comber, S. D. W., Conrad, A. U., Howcroft, J. & Zaman, N. 2000. Inputs, monitoring and fate modelling of antifouling biocides in UK estuaries. *Marine Pollution Bulletin*, **40,** 898-905.

Boyden, C. R. 1975. Distribution of some trace metals in Poole Harbour, Dorset. *Marine Pollution Bulletin*, **6,** 180-187.

Braman, R. S. & Foreback, C. C. 1973. Methylated forms of arsenic in the environment. *Science*, **182,** 1247-1249.

Braune, B. M. 1987. Comparison of total mercury levels in relation to diet and moult for nine species of marine birds. *Archives of Environmental Contamination and Toxicology*, **16,** 217-224.

Braune, B. M., Donaldson, G. M. & Hobson, K. A. 2001. Contaminant residues in seabird eggs from the Canadian Arctic. Part I. Temporal trends 1975-1998. *Environmental Pollution*, **114,** 39-54.

Braune, B. M., Donaldson, G. M. & Hobson, K. A. 2002. Contaminant residues in seabird eggs from the Canadian Arctic. II. Spatial trends and evidence from stable isotopes for intercolony differences. *Environmental Pollution*, **117,** 133-145.

Braune, B. M. & Gaskin, D. E. 1987a. Mercury levels in Bonaparte's gull (*Larus philadelphia*) during autumn molt in the Quoddy region, New Brunswick, Canada. *Archives of Environmental Contamination and Toxicology*, **16,** 539-549.

Braune, B. M. & Gaskin, D. E. 1987b. A mercury budget for the Bonaparte's Gull during autumn moult. *Ornis Scandinavica*, **18,** 244-250.

Braune, B. M. & Simon, M. 2004. Trace elements and halogenated organic compounds in Canadian Arctic seabirds. *Marine Pollution Bulletin*, **48,** 986-1008.

Bressman, R. B., Miles, R. D., Comer, C. W., Wilson, H. R. & Butcher, G. D. 2002. Effect of dietary supplementation of vanadium in commercial egg-type laying hens. *Journal of Applied Poultry Research*, **11,** 46-53.

Brewer, P. A., Dennis, I. A. & Macklin, M. G. 2005. The use of geomorphological mapping and modelling for identifying land affected by metal contamination on river floodplains. In: *Defra R&D Report SP 0525*.

British Gypsum. 2009a. Press Releases: Kirkby Thore Landfill: http://www.british-gypsum.com/press/press_releases/kirkby_thore_landfill.aspx.

British Gypsum. 2009b. Kirkby Thore Landfill: http://www.british-gypsum.com/contact_us/locations/kirkby_thore.aspx.

Brown, K. M. & Morris, R. D. 1996. From tragedy to triumph: renesting in Ring-billed Gulls. *The Auk*, **113,** 23-31.

Brunton, D. H. 1997. Impacts of predators: center nests are less successful than edge nests in a large nesting colony of Least Terns. *The Condor*, **99,** 372-380.

Bryan, A. L., Hopkins, W. A., Baionno, J. A. & Jackson, B. P. 2003. Maternal transfer of contaminants to eggs in Common Grackles (*Quiscalus quiscala*) nesting on coal fly ash basins. *Archives of Environmental Contamination and Toxicology*, **45,** 273-277.

Bryan, G. W. & Langston, W. J. 1992. Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: a review. *Environmental Pollution*, **76,** 89-131.

BTO. 2007. The Population Status of Birds in the UK: Birds of Conservation Concern 2002-2007 - Amber List Species
http://www.bto.org/psob/amberlist.htm#amber: British Trust for Ornithology.

Burger, J. 1976. Nest density of the Black-headed gull in relation to vegetation. *Bird Study*, **23,** 27-32.

Burger, J. 1993. Metals in avian feathers: bioindicators of environmental pollution. *Reviews in Environmental Toxicology*, **5,** 203-311.

Burger, J. 1994. Heavy metals in avian eggshells: another excretion method. *Journal of Toxicology and Environmental Health*, **41,** 207-220.

Burger, J. 1995. Heavy metal and selenium levels in feathers of herring gull (*Larus argentatus*): Differences due to year, gender, and age at Captree, Long Island. *Environmental Monitoring and Assessment*, **38,** 37-50.

Burger, J. 1996. Heavy metal and selenium levels in feathers of Franklin's Gulls in interior North America. *The Auk*, **113,** 399-407.

Burger, J. 2002. Food chain differences affect heavy metals in bird eggs in Barnegat Bay, New Jersey. *Environmental Research, Section A*, **90,** 33-39.

Burger, J., Bowman, R., Woolfenden, G. E. & Gochfeld, M. 2004. Metal and metalloid concentrations in the eggs of threatened Florida scrub-jays in suburban habitat from south-central Florida. *Science of the Total Environment*, **328,** 185-193.

Burger, J. & Gochfeld, M. 1981. Nest site selection by Kelp Gulls in Southern Africa. *Condor*, **83,** 243-251.

Burger, J. & Gochfeld, M. 1985. Comparisons of nine heavy metals in salt gland and liver of great scaup (*Aythya marila*), black duck (*Anas rubripes*), and mallard (*A. platyrynches*). *Comparative Biochemistry and Physiology* **81C,** 287-292.

Burger, J. & Gochfeld, M. 1987. Nest-site selection by Mew Gulls (*Larus canus*): a comparison of marsh and dry-land colonies. *Wilson Bulletin*, **99,** 673-687.

Burger, J. & Gochfeld, M. 1988. Metals in tern eggs in a New Jersey estuary: a decade of change. *Environmental Monitoring and Assessment*, **11,** 127-135.

Burger, J. & Gochfeld, M. 1991. Cadmium and lead in common terns (Aves: *Sterna hirundo*): relationships between levels in parents and eggs. *Environmental Monitoring and Assessment*, **16,** 253-258.

Burger, J. & Gochfeld, M. 1992a. Trace element distribution in growing feathers: additional excretion in feather sheaths. *Archives of Environmental Contamination and Toxicology*, **23,** 105-108.

Burger, J. & Gochfeld, M. 1992b. Heavy metal and selenium concentrations in black skimmers (*Rynchops niger*). Gender differences. *Archives of Environmental Contamination and Toxicology*, **23,** 431-434.

Burger, J. & Gochfeld, M. 1993. Lead and cadmium accumulation in eggs and fledgling seabirds in the New York bight. *Environmental Toxicology and Chemistry*, **12,** 261-267.

Burger, J. & Gochfeld, M. 1994. Predation and effects of humans on island-nesting seabirds. In: *Seabirds on Islands: Threats, Case Studies and Action Plans* (Ed. by Nettleship, D. N., Burger, J. & Gochfeld, M.), pp. 39-67. Cambridge, UK: Birdlife International.

Burger, J. & Gochfeld, M. 1995a. Heavy metals and selenium concentrations in eggs of herring gulls (*Larus argentatus*): temporal differences from 1989 to 1994. *Archives of Environmental Contamination and Toxicology*, **29,** 192-197.

Burger, J. & Gochfeld, M. 1995b. Growth and behavioural effects of early postnatal chromium and manganese exposure in Herring Gull (*Larus argentatus*) chicks. *Pharmacology Biochemistry and Behavior*, **50,** 607-612.

Burger, J. & Gochfeld, M. 1996. Heavy metal and selenium levels in birds at Agassiz National Wildlife Refuge, Minnesota: food chain differences. *Environmental Monitoring and Assessment*, **43,** 267-282.

Burger, J. & Gochfeld, M. 1997. Heavy metal and selenium concentrations in feathers of egrets from Bali and Sulawesi, Indonesia. *Archives of Environmental Contamination and Toxicology*, **32,** 217-221.

Burger, J. & Gochfeld, M. 1999. Heavy metals in Franklin's gull tissues: age and tissue differences. *Environmental Toxicology and Chemistry*, **18,** 673-678.

Burger, J. & Gochfeld, M. 2000a. Metal levels in feathers of 12 species of seabirds from Midway Atoll in the northern Pacific Ocean. *Science of the Total Environment*, **257,** 37-52.

Burger, J. & Gochfeld, M. 2000b. Metals in albatross feathers from Midway Atoll: Influence of species, age, and nest location. *Environmental Research Section A*, **82,** 207-221.

Burger, J. & Gochfeld, M. 2000c. Effects of lead on birds (Laridae): A review of laboratory and field studies. *Journal of Toxicology and Environmental Health, Part B*, **3,** 59-78.

Burger, J. & Gochfeld, M. 2002. Effects of chemicals and pollution on seabirds. In: *Biology of Marine Birds* (Ed. by Schreiber, E. A. & Burger, J.), pp. 485-525. Boca Raton: CRC Press LLC.

Burger, J. & Gochfeld, M. 2004. Marine birds as sentinels of environmental pollution. *EcoHealth*, **1,** 263-274.

Burger, J., Laska, M. & Gochfeld, M. 1993. Metal concentrations in feathers of birds from Papua New Guinea forests: evidence of pollution. *Environmental Toxicology and Chemistry*, **12,** 1291-1296.

Burger, J., Nisbet, I. C. T. & Gochfeld, M. 1992. Metal levels in regrown feathers: assessment of contamination on the wintering and breeding grounds in the same individuals. *Journal of Toxicology and Environmental Health*, **37,** 363-374.

Burges, D. 2000. Ornithology of the Solent. In: *Solent Science - A Review* (Ed. by Collins, M. & Ansell, K.), pp. 261-270. London: Elsevier.

Butler, R. G. & Trivelpiece, W. 1981. Nest spacing, reproductive success, and behaviour of the Great Black-backed Gull (*Larus marinus*). *The Auk*, **98,** 99-107.

CAA. 2009. UK Airport Terminal Passengers 1999-2009. UK Civil Aviation Authority.

Cahill, T. M., Anderson, D. W., Elbert, R. A., Perley, B. P. & Johnson, D. R. 1998. Elemental profiles in feather samples from a mercury-contaminated lake in central California. *Archives of Environmental Contamination and Toxicology*, **35,** 75-81.

Cain, B. W. & Pafford, E. A. 1981. Effects of dietary nickel o survival and growth of mallard ducklings. *Archive of Environmental Contamination and Toxicology*, **10,** 737-745.

Callahan, M. A. 1979. *Water-related fate of 129 priority pollutants, Volumes I and II. EPA 440/4-79-029a and 029b*. Washington, D.C.: US Environmental Protection Agency Office of Water Planning and Standards.

Callahan, M. A., Ehreth, D. J. & Levins, P. L. 1979. Sources of toxic pollutants found in influent to sewage treatment plants. *Proceedings of the National Conference on Municipal Sludge Management*, **8th,** 55-61.

Carbonell, G. & Tarazona, J. V. 1994. Toxicokinetics of copper in rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology*, **29,** 213-221.

Carey, C. 1980. Adaptation of the avian egg to high altitude. *American Zoologist*, **20,** 449-459.

Carey, C. 1996. Female reproductive energies. In: *Avian Energetics and Nutritional Ecology* (Ed. by Carey, C.), pp. 324-369. New York: International Thomson Publishing.

Cave, R. R., Andrews, J. E., Jickells, T. & Coombes, E. G. 2005. A review of sediment contamination by trace metals in the Humber catchment and estuary, and the implications for future estuary water quality. *Estuarine, Coastal and Shelf Science*, **62,** 547-557.

Chiou, P. W., Chen, K.-L. & Yu, B. 1997. Toxicity, tissue accumulation and residue in egg and excreta of copper in laying hens. *Animal Feed Science Technology*, **67,** 49-60.

Cicchella, D., De Vivo, B., Lima, A., Albanese, S., McGill, R. A. R. & Parrish, R. R. 2008. Heavy metal pollution and Pb isotopes in urban soils of Napoli, Italy. *Geochemistry: exploration, environment, analysis*, **8,** 103-112.

CIWEM. 2004. The Water Framework Directive: integrating approaches to diffuse pollution. London: Chartered Institute of Water and Environmental Management (CIWEM).

Clark, T. P., Norstrom, R. J., Fox, G. A. & Won, H. T. 1987. Dynamics of organochlorines in herring gulls (Larus argentatus): II. A two compartment model and data for ten compounds. *Environmental Toxicology and Chemistry*, **6,** 547-559.

Clarkson, T. W. 1986. Effects - general principles underlying the toxic action of metals. In: *Handbook of the Toxicology of Metals* (Ed. by Friberg, L., Norberg, G.F. and Vouk, V.B.), pp. 128-148. Amsterdam: Elsevier

Cody, M. L. 1985. An introduction to habitat selection in birds. In: *Habitat Selection in Birds* (Ed. by Cody, M.), pp. 3-56. New York: Academic Press.

Colenutt, A. J. 2005. Notes on Lymington and Keyhaven saltmarshes and inter-tidal mudflats for the Western Solent Coastal Defence Strategy Study. New Forest District Council.

Comber, S. D. W., Gardner, M. J. & Boxall, A. B. A. 2002. Survey of four marine antifoulant constituents (copper, zinc, diuron and Irgarol 1051) in two UK estuaries. *Journal of Environmental Monitoring*, **4,** 417-425.

Comber, S. D. W. & Gunn, A. M. 1996. Heavy metals entering sewage treatment works from domestic sources. *Journal of CIWEM*, **10,** 137-142.

Confer, J. L. 2003. Effects of vegetation, interspecific competition, and brood parasitism on Golden-winged Warbler (*Vermivora chrysoptera*) nesting success. *The Auk*, **120,** 138-144.

Connell, D. W., Fung, C. N., Minh, T. B., Tanabe, S., Lam, P. K. S., Wong, B. S. F., Lam, M. H. W., Wong, L. C., Wu, R. S. S. & Richardson, B. J. 2003. Risk to breeding success of fish-eating Ardeids due to persistent organic contaminants in Hong Kong: evidence from organochlorine compounds in eggs. *Water Research*, **37,** 459-467.

Cooke, F., Rockwell, R. F. & Lank, D. B. 1995. *The snow geese of La Pérouse Bay*. Oxford University Press.

Cory-Slechta, D. A., Weiss, B. & Cox, C. 1983. Delayed behavioural toxicity of lead with increasing exposure concentration. *Toxicology and Applied Pharmacology*, **71,** 342-352.

Cotton, F. A., Wilkinson, G., Murillo, C. A. & Bochmann, M. 1999. *Advanced Inorganic Chemistry, 6th Edition*. Chichester: John Wiley & Sons.

Coulson, J. C. 1963. Egg size and shape in the kittiwake *Rissa tridactyla* and their use in estimating age composition of populations. *Proceedings of the Royal Zoological Society of London*, **140,** 211-227.

Coulson, J. C., Duncan, N. & Thomas, C. 1982. Changes in the breeding biology of the herring gull (*Larus argentatus*) induced by reduction in the size and density of the colony. *Journal of Animal Ecology*, **51,** 739-756.

Coulson, J. C. & Porter, J. M. 1985. Reproductive success of the kittiwake *Rissa tridactyla*: the roles of clutch size, chick growth rates and parental quality. *Ibis*, **127,** 450-466.

Coulson, J. C. & White, E. 1958. The effect of age on the breeding biology of the kittiwake, *Rissa tridactyla*. *Ibis*, **100,** 40-51.

Cowes Harbour Authority. 2008. Cowes Harbour: Marina Berths. www.cowes.co.uk.

CPRE & Natural England. 2010. Green Belts: a greener future - South West Hampshire and South East Dorset Green Belt. Joint report by the Campaign to Protect Rural England (CPRE) and Natural England.

Craig, J. R., Rimstidt, J. D., Bonnaffon, C. A., Collins, T. K. & Scanlon, P. F. 1999. Surface water transport of lead at a shooting range. *Bulletin of Environmental Contamination and Toxicology*, **63**.

Cramp, S. 1983. *Larus ridibundus* Black-headed Gull. In: *Handbook of the Birds of Europe and the Middle East and North Africa - The Birds of the Western Palearctic* (Ed. by Cramp, S.): RSPB & Oxford University Press.

Cramp, S. & Simmons, K. E. L. 1983. *Handbook of the birds of Europe, the Middle East and North Africa*. New York: Oxford University Pres.

Crans, D. C., Aim, S. S. & Keramidas, A. D. 1998. Chemistry of relevance to vanadium in the environment. In: *Vanadium in the Environment. Part 1: Chemistry and Biochemistry* (Ed. by Nriagu, J. O.), pp. 325-264. New York: John Wiley and Sons.

Crown Estate. 2004. Marinas and Moorings: Hamble. http://www.thecrownestate.co.uk/hamble.

Croxall, J. P., Evans, P. G. H. & Schreiber, R. W. 1984. Status and conservation of the World's seabirds. In: *ICBP Technical Publication No. 2*. Cambridge.

Cukrowska, E. M., Nsengimana, H. & Chimuka, L. 2007. Speciation of alkyllead in aqueous samples with application of liquid membrane probe for extraction and preconcentration *Journal of Separation Science*, **30,** 2754-2759.

Cundy, A. B. & Croudace, I. W. 1995. Physical and chemical associations of radionuclides and trace metals in estuarine sediments: and example from Poole Harbour, Southern England. *Journal of Environmental Radioactivity*, **29,** 191-211.

Custer, T. W., Franson, J. C. & Pattee, O. H. 1984. Tissue lead distribution and hematologic effects in American kestrels (*Falco sparverius* L.) fed biologically incorporate lead. *Journal of Wildlife Diseases*, **20,** 39-43.

Custer, T. W., Hensler, G. L. & Kaiser, T. E. 1983. Clutch size, reproductive success, and organochlorine contaminants in Atlantic coast Black-crowned Night-herons. *Auk*, **100,** 699-710.

Custer, T. W. & Hohman, W. L. 1994. Trace elements in canvasbacks (*Aythya valisineria*) wintering in Louisiana, USA, 1987–1988. *Environmental Pollution*, **84,** 253-259.

Dannecker, W., Au, M. & Stechmann, H. 1990. Substance load in rainwater runoff from different streets in Hamburg. *Science of the Total Environment*, **93,** 385-392.

Darling, F. F. 1938. *Bird Flocks and the Breeding Cycle*. Cambridge: Cambridge University Press.

Daunt, F., Wanless, S., Harris, M. P. & Monaghan, P. 1999. Experimental evidence that age-specific reproductive success is independent of environmental effects. *Proceedings of the Royal Society London: Series B*, **266,** 1489-1493.

Dauwe, T., Bervoets, L., Blust, R. & Eens, M. 2002b. Tissue levels of lead in experimentally exposed zebra finches (*Taeniopygia guttata*) with particular attention on the use of feathers as biomonitors. *Archives of Environmental Contamination and Toxicology*, **42,** 88-92.

Dauwe, T., Bervoets, L., Blust, R., Pinxten, R. & Eens, M. 1999. Are eggshells and egg contents of Great and Blue Tits suitable as indicators of heavy metal pollution? *Belgian Journal of Zoology*, **129,** 439-447.

Dauwe, T., Bervoets, L., Blust, R., Pinxten, R. & Eens, M. 2000. Can excrement and feathers of nestling songbirds be used as biomonitors for heavy metal pollution? *Archives of Environmental Contamination and Toxicology*, **39,** 541-546.

Dauwe, T., Bervoets, L., Janssens, E., Pinxten, R., Blust, R. & Eens, M. 2002a. Great and blue tit feathers as biomonitors for heavy metal pollution. *Ecological Indicators*, **1,** 227-234.

Dauwe, T., Bervoets, L., Pinxten, R., Blust, R. & Eens, M. 2003. Variation of heavy metals within and among feathers of birds of prey: effects of molt and external contamination. *Environmental Pollution*, **124,** 429-436.

Dauwe, T., Janssens, E., Bervoets, L., Blust, R. & Eens, M. 2004. Relationships between metal concentrations in great tit nestlings and their environment and food. *Environmental Pollution*, **131,** 373-380.

Dauwe, T., Janssens, E., Bervoets, L., Blust, R. & Eens, M. 2005. Heavy-metal concentrations in female laying great tits (*Parus major*) and their clutches. *Archives of Environmental Contamination and Toxicology*, **49,** 249-256.

Davies, C. A. L., Tomlinson, K. & Stephenson, T. 1991. Heavy metals in River Tees estuary sediments. *Environmental Technology*, **12,** 961-972.

Davis, A. P. & Burns, M. 1999. Evaluation of lead concentration in runoff from painted structures. *Water Research*, **33,** 2949-2958.

Davis, A. P., Shokouhian, M. & Ni, S. 2001. Loading estimates of lead, copper, cadmium, and zinc in urban runoff from specific sources *Chemosphere*, **44,** 997-1009.

Davis, E. G., Butcher, G. D. & Miles, R. D. 1995. Characterizing the time related detrimental effects of dietary vanadium of performance and immune response of commercial egg-type laying hens. *Poultry Science*, **74,** 61.

Davis, J. W. F. 1975. Age, egg size, and breeding success in the Herring gull Larus argentatus. *Ibis*, **117,** 460-473.

Davis, T. A. & Ackerman, R. A. 1987. Effects of increased water loss on growth and water content of the chick embryo. *Journal of Experimental Zoology* **Supplement 1,** 357-364.

de Juana, E. 1984. The status and conservation of seabirds in the Spanish Mediterranean. In: *Status and conservation of the World's Seabirds* (Ed. by Croxall, J. P., Evans, P. G. H. & Schreiber, R. W.), pp. 347-370. Cambridge, UK: International Council for Birds Preservation.

Decuypere, E., Helsen, J., Gorp, S. V. & Verheyen, G. 1988. The use of high zinc diets as forced moulting method: effect on Zn uptake and egg Zn content. *Archives fur Gefluegelkunde*, **52,** 245-251.

Denton, G. R. W., Wood, H. R., Concepcion, L. P., Siegrist, H. G., Eflin, V. S., Narcis, D. K. & Pangelinan, G. T. 1997. Analysis of in-place contaminants in marine sediments from four harbor locations on Guam: A Pilot Study, Technical Report No. 87, . Mangilao, Guam: University of Guam; Water and Environmental Research Institute of the Western Pacific.

Dexheimer, M. & Southern, W. E. 1974. Breeding success relative to nest location and density in Ring-billed Gull colonies. *Wilson Bulletin*, **86,** 288-290.

Diaz, G. J., Julian, R. J. & Squires, E. J. 1994. Lesions in broiler chickens following experimental intoxication with cobalt. *Avian Diseases*, **38,** 308-316.

Diller, F. 2004. South Beach diet. *American Scientist*, **92,** 1.

Dorset AONB. 2009. Farming & agricultural land management. Dorchester: Dorset Area of Outstanding Natural Beauty.

Drake, W. 2007. Poole Harbour Aquatic Management Plan 2006. Poole Harbour Steering Group: Borough of Poole, Dorset County Council, Environment Agency, Natural England, Poole Harbour Commissioners, Purbeck District Council, Southern Sea Fisheries District Committee, Wessex Water Services Ltd.

Dunham, K. C. 1948. *Geology of the North Pennines Orefield, Tyne to Stainmore*. London: Memoir of the Geological Society of the UK; HMSO.

Dunham, K. C. 1990. *Geology of the Northern Pennines Orefield, Vol. 1, Tyne to Stainmore*. Economic Memoir of the British Geological Society, 2nd Edition, Sheets 13, 19, 24, 25, 26, 31 and 32.

Durham County Council. 2004. Agricultural Land Classification: North Pennines. Natural England.

Dyke, P. H., Foan, C. & Fiedlerc, H. 2003. PCB and PAH releases from power stations and waste incineration processes in the UK *Chemosphere*, **50,** 469-480.

EA. 2007. Lead and its compounds. In: *Pollution Inventory*: Environment Agency.

EAI. 2010. Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Elemental Analysis Inc.: http://www.elementalanalysis.com/services_icp.asp.

Eastin, W. C., Jr. & O'Shea, T. J. 1981. Effects of dietary nickel on mallards. *Journal of Toxicology and Environmental Health*, **7,** 883-892.

EC. 2006. Environment fact sheet: protecting and conserving the marine environment. European Commission.

Eden District Council. 2008. Towns in Eden: http://www.eden.gov.uk/your-community/towns-in-eden/.

Edens, F. W., Benton, E., Bursian, S. J. & Morgan, G. W. 1976. Effects of dietary lead on reproductive performance in japanese quail, *Coturnix coturnix japonica*. *Toxicology and Applied Pharmacology*, **38,** 307-314.

Eens, M., Pinxten, R., Verheyen, R. F., Blust, R. & Bervoets, L. 1999. Great and blue tits as indicators of heavy metal contamination in terrestrial ecosystems. *Ecotoxicology and Environmental Safety*, **44,** 81-85.

Eeva, T. & Lehikoinen, E. 1995. Egg shell quality, clutch size and hatching success of the great tit (*Parus major*) and the pied flycatcher (*Ficedula hypoleuca*) in an air pollution gradient. *Oecologia*, **102,** 312-323.

EHC. 1989. EHC 85: Lead and lead compounds. In: *Environmental Health Criteria*.

EHC. 2001. EHC 224: Arsenic and arsenic compounds. In: *Environmental Health Criteria*.

Eising, C. M., Eikenaar, C., Schwabl, H. & Groothuis, T. G. G. 2001. Maternal androgens in black-headed gull (*Larus ridibundus*) eggs: consequences for chick development. *Proceedings of the Royal Society London: Biological Sciences*, **268,** 839-846.

Eisler, R. 1985a. Cadmium hazards to fish, wildlife, and invertebrates: a synoptic review. In: *Biological Report No. 2*. Washington, DC: US Fish and Wildlife Service.

Eisler, R. 1985b. Selenium hazards to fish, wildlife, and invertebrates: a synoptic review. In: *Biological Report No. 5*. Washington, DC: US Fish and Wildlife Service.

Eisler, R. 1988a. Lead hazards to fish, wildlife, and invertebrates: a synoptic review. In: *Biological Report No. 14*. Washington, DC: US Fish and Wildlife Service.

Eisler, R. 1988b. Arsenic hazards to fish, wildlife, and invertebrates: a synoptic review. In: *Biological Report No. 12*. Washington, DC: US Fish and Wildlife Service.

Eisler, R. 1993. Zinc hazards to fish, wildlife, and invertebrates: a synoptic review. In: *Biological Report No. 26*. Washington, DC: US Fish and Wildlife Service.

Eisler, R. 1994. A review of arsenic hazards to plants and animals with emphasis on fishery and wildlife. In: *Arsenic in the environment: Part II: Human health and ecosystem effects* (Ed. by Nriagu, J. O.), pp. 185-259. New York: John Wiley & Sons.

Eisler, R. 1998a. Nickel hazards to fish, wildlife, and invertebrates: a synoptic review. In: *Biological Report No. 34*. Washington, DC: US Fish and Wildlife Service.

Eisler, R. 1998b. Copper hazards to fish, wildlife, and invertebrates: a synoptic review. In: *Biological Report No. 33*. Washington, DC: US Fish and Wildlife Service.

El-Begearmi, M. M., Sunde, M. L. & Ganther, H. E. 1977. A mutual protective effect of mercury and selenium in Japanese quail. *Poultry Science*, **56,** 313-322.

Emerson, S., Cranston, R. E. & Liss, P. S. 1979. Redox species in a reducing fjord: equilibrium and kinetic considerations. *Deep Sea Research*, **26A,** 859-878.

Enderson, J. H. & Berger, D. D. 1970. Pesticides: eggshell thinning and lowered production of young in prairie falcons. *Bioscience*, **20,** 355-356.

English Nature. 1998. Solent and Poole Bay Natural Area profile. In: *English Nature Natural Area Profiles* (Ed. by Langslow, D.): English Nature (now Natural England).

English Nature. 2004. Moor House-Upper Teesdale National Nature Reserve. Stocksfield, Northumberland: English Nature (now Natural England).

Environment Agency. 2006. New Forest Catchment Flood Management Plan - Scoping Report. Environment Agency: http://www.environment-agency.gov.uk/static/documents/Research/2nf_1452450.pdf.

Environment Agency. 2008. Assessment of metal mining-contaminated river sediments in England and Wales. Science Report SC030136/SR4. Bristol: Environment Agency.

Environment Agency. 2009a. What's In Your Backyard? http://maps.environment-agency.gov.uk/wiyby/wiybyController?ep=maptopics&lang=_e.

Environment Agency. 2009b. Pollution: Acid rain. http://www.environment-agency.gov.uk/research/library/data/34337.aspx#deposit.

Environment Agency. 2010. How do we regulate landfill sites? http://www.environment-agency.gov.uk/homeandleisure/37825.aspx.

Eriksen, K. D., Andersen, T., Sternersen, J. & Andersen, R. A. 1990. Cystolic binding of Cd, Cu, Zn and Ni in four polychaete species. *Comparative Biochemistry and Physiology*, **95C,** 111-115.

Erry, B. V., Macnair, M. R., Meharg, A. A., Shore, R. F. & Newton, I. 1999. Arsenic residues in predatory birds from an area of Britain with naturally and anthropogenically elevated arsenic levels. *Environmental Pollution*, **106,** 91-95.

Euro Gypsum. 2007. Factsheet on: What is Gypsum? : Environment and Raw Material Commitee Euro Gypsum.

Fair, J. M. & Myers, O. B. 2002. Early reproductive success of western bluebirds and ash-throated flycatchers: a landscape-contaminant perspective. *Environmental Pollution*, **118,** 321-330.

Farag, A. M., Woodward, D. F., Goldstein, J. N., Brumbaugh, W. & Meyer, J. S. 1998. Concentrations of metal associated with mining waste in sediments, biofilm, benthic macroinvertebrates, and fish from the Coeur d'Alene River basin, Idaho. *Archive of Environmental Contamination and Toxicology*, **34,** 119-127.

Feare, C. J. 1976a. The exploitation of sooty tern eggs in the Seychelles. *Biological Conservation*, **10,** 169-181.

Feare, C. J. 1976b. The breeding of the sooty tern *Sterna fuscata* in the Seychelles and the effects of experimental removal of its eggs. *Journal of Zoology*, **179,** 317-360.

Field, A. 2005. *Discovering Statistics Using SPSS*. London: SAGE Publications.

Finkler, M. S., Van Orman, J. B. & Sotherland, P. R. 1998. Experimental manipulation of egg quality in chickens: influence of albumen and yolk on the size and body composition of near-term embryos in a precocial bird. *Journal of Comparative Physiology B*, **168,** 17-24.

Florence, T. M. & Batley, G. E. 1980. Chemical speciation in natural waters. *CRC Critical Reviews in Analytical Chemistry*, **9,** 219-296.

Focardi, S., Fossi, C., Lambertini, M., Leonzio, C. & Massi, A. 1988. Long term monitoring of pollutants in eggs of yellow-legged herring gulls from Capraia Island (Tuscan Archipelago). *Environmental Monitoring and Assessment*, **10,** 43-50.

Forsyth, D. J., Martin, P. A., De Smet, K. D. & Riske, M. E. 1994. Organochlorine contaminants and eggshell thinning in grebes from prairie Canada. *Environmental Pollution*, **85,** 51-58.

Forsyth, D. S., Marshall, W. D. & Collette, M. C. 1985. Interaction of alkyllead salts with avian eggs. *Journal of Environmental Science and Health*, **20A,** 177-191.

Freedman, B. 1995. *Environmental Ecology: the ecological effects of pollution, disturbance, and other stresses. Second Edition*. London: Academic Press.

Furness, R. W. 1983. Variations in size and growth of great skua *Catharacta skua* chicks in relation to adult age, hatching date, egg volume, brood size and hatching sequence. *Journal of Zoology*, **199,** 101-116.

Furness, R. W. 1993. Birds as monitors of pollutants. In: *Birds as Monitors of Environmental Change* (Ed. by Furness, R. W. G., J.J.D.), pp. 86-143. London: Chapman & Hall.

Furness, R. W. 1996. Cadmium in birds. In: *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations* (Ed. by Beyer, W. N., Heinz, G. H. & Redmon-Norwood, A. W.), pp. 389-404. Boca Raton, Florida: Lewis Publishers, CRC Press.

Furness, R. W. & Camphuysen, C. J. 1997. Seabirds as monitors of the marine environment. *ICES Journal of Marine Science*, **54,** 726-737.

Furness, R. W., Greenwood, J. J. D. & Jarvis, P. J. 1993. Can birds be used to monitor the environment? In: *Birds as Monitors of Environmental Change* (Ed. by Furness, R. W. G., J.J.D.), pp. 1-41. London: Chapman & Hall.

Furness, R. W., Muirhead, S. J. & Woodburn, M. 1986. Using bird feathers to measure mercury in the environment: relationships between mercury content and moult. *Marine Pollution Bulletin*, **17,** 27-30.

Furness, R. W. & Rainbow, P. S. 1990. *Heavy Metals in the Marine Environment*. Boca Raton, Florida: CRC Press.

García-Borboroglu, P. & Yorio, P. 2004. Habitat requirements and selection by Kelp Gulls (*Larus dominicanus*) in Central and Northern Patagonia, Argentina. *The Auk*, **121,** 243-252.

Gasaway, W. C. & Buss, I. O. 1972. Zinc toxicity in the mallard duck. *Journal of Wildlife Management*, **36,** 1107-1117.

Gerritse, R. G. 1981. Trace element mobility in soils. In: *Proceedings for conference: Heavy Metals in the Environment*, pp. 180-185. Amsterdam.

Gibbons, D. W., Reid, J. B. & Chapman, R. A. 1993. *The New Atlas of Breeding Birds in Britain & Ireland: 1988-1991*. London, UK: T. & A.D. Poyser.

Gilani, S. H. & Alibhai, Y. 1990. Teratogenicity of metals to chick embryos. *Journal of Toxicology and Environmental Health*, **30,** 23-31.

Glutz von Blotzenheim, U. N. & Bauer, K. M. 1982. *Handbuch der Vögel Mitteleuropas*. Wiesbaden: Akademische Verlagsgellshaft.

Gochfeld, M. 1978. Colony and nest site selection by Black Skimmers. In: *Proceedings of the First Conference of the Colonial Waterbird Group*.

Gochfeld, M. 1997. Spatial patterns in a bioindicator: heavy metal and selenium concentration in eggs of herring gulls (*Larus argentatus*) in the New York Bight. *Archives of Environmental Contamination and Toxicology*, **33,** 63-70.

Gochfeld, M., Belant, J. L., Shukla, T., Benson, T. & Burger, J. 1996. Heavy metals in laughing gulls: gender, age and tissue differences. *Environmental Toxicology and Chemistry*, **15,** 2275-2283.

Gochfeld, M. & Burger, J. 1998. Temporal trends in metal levels in eggs of the endangered Roseate tern (*Sterna dougallii*) in New York. *Environmental Research Section A*, **77,** 36-42.

Goede, A. A. & de Bruin, M. 1984. The use of bird feather parts as a monitor for metal pollution. *Environmental Pollution Series B*, **8,** 281-298.

Goede, A. A. & de Bruin, M. 1986. The use of feathers for indicating heavy metal pollution. *Environmental Monitoring and Assessment*, **7,** 249-256.

González, J. A. 1999. Effects of harvesting of waterbirds and their eggs by native people in the Northeastern Peruvian Amazon. *Waterbirds*, **22,** 217-224.

González, M. J. & Hiraldo, F. 1988. Organochlorine and heavy metal contamination in the eggs of the Spanish Imperial Eagle (*Aquila (heliaca) adalberti*) and accompanying changes in eggshell morphology and chemistry. *Environmental Pollution*, **51,** 241-258.

Gorke, M. & Brandl, R. 1986. How to live in colonies: spatial foraging strategies of the black-headed gull. *Oecologia*, **70,** 288-290.

Gosselin, R. E., Smith, R. P. & Hodge, H. C. 1984. *Clinical Toxicity of Commercial Products*. Baltimore: Williams and Wilkins.

Gozzard, E., Mayes, W. M., Morrison, M. I. & Jarvis, A. P. 2006. Mine water management at the catchment scale: case studies from North East England. In: *Proceedings of the Seventh International Conference of Acid Rock Drainage (ICARD)* (Ed. by Barnishel, R. I.), pp. 665-675. St Louis, Missouri, USA: American Society of Mining & Reclamation (ASMR).

Grandjean, P. 1976. Possible effect of lead on egg-shell thickness in Kestrels 1874–1974. *Bulletin of Environmental Contamination and Toxicology* **16,** 101-106.

Grey, L. E. & Laskey, J. W. 1980. Multivariate analysis of the effects of manganese on the reproductive physiology and behaviour of the male house mouse. *Journal of Toxicology and Environmental Health*, **6,** 861-867.

Guitart, R., Torra, M., Cerradelo, S., Puig Casado, P., Mateo, R. & To Figueras, J. 1994. Pb, Cd, As, and Se concentrations in livers of dead wild birds from the Ebro Delta, Spain. *Bulletin of Environmental Contamination and Toxicology*, **52,** 523-529.

Guthová, Z. 1993. Variations in reproduction parameters of Black-headed Gulls (*Larus ridibundus*) living in different conditions in the Czech and Slovak Republics. *Environmental Conservation*, **20,** 347-351.

Haegele, M. A. & Tucker, R. K. 1974. Effects of 15 common environmental pollutants on eggshell thickness in mallards and coturnix. *Bulletin of Environmental Contamination and Toxicology*, **11,** 98-102.

Haegele, M. A., Tucker, R. K. & Hudson, R. H. 1974. Effects of dietary mercury and lead on eggshell thickness in mallards. *Bulletin of Environmental Contamination and Toxicology*, **8,** 5-11.

Hafez, Y. S. M. & Kratzer, F. H. 1976. The effect of diet on the toxicity of vanadium. *Poultry Science*, **55,** 918-922.

Hall, L. & Anderson, R. 1999. A deterministic ecological risk assessment for copper in European saltwater environments. *Marine Pollution Bulletin*, **26,** 365-373.

Hamble Interactive. 2009. BP in Hamble. http://www.hamble.net/BP2.html.

Hamilton, E. I. 1994. The geobiochemistry of cobalt. *The Science of the Total Environment*, **150,** 7-39.

Harms, R. H. & Hussein, S. M. 1993. Variations in yolk:albumen ratio in hen eggs from commercial flocks. *Journal of Applied Poultry Research*, **2,** 166-170.

Harr, J. R. 1978. Biological effects of selenium. In: *Toxicity of Heavy Metals in the Environment, Part 1* (Ed. by Oehme, F. W.), pp. 393-426. New York: Marcel Dekker.

Harrison, R. M. & Laxen, D. P. H. 1981. *Lead Pollution: Causes and Control*. New York: Chapman & Hall.

Haynes, A. M. 1987. Human exploitation of seabirds in Jamaica. *Biological Conservation*, **41,** 99-124.

Heaney, V. & Monaghan, P. 1995. A within-clutch trade-off between egg production and rearing in birds. *Proceedings of the Royal Society London: Biological Sciences*, **261,** 361-365.

Heinz, G. 1974. Effects of low dietary levels of methyl mercury on mallard reproduction. *Bulletin of Environmental Contamination and Toxicology*, **11,** 386-392.

Heinz, G. H. 1996. Selenium in birds. In: *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations* (Ed. by Beyer, W. N., Heinz, G. H. & Redmon-Norwood, A. W.), pp. 447-458. Boca Raton, Florida: Lewis Publishers, CRC Press.

Heinz, G. H., Hoffman, D. J. & Gold, L. G. 1988. Impaired reproduction of mallards fed an organic form of selenium. *Journal of Wildlife Management*, **53,** 418-428.

Heinz, G. H., Hoffman, D. J., Krynitsky, A. J. & Weller, D. M. G. 1987. Reproduction in mallards fed selenium. *Environmental Toxicology and Chemistry*, **6,** 423-433.

Heinz, G. H., Hoffman, D. J., Sileo, L., Audet, D. J. & Lecaptain, L. J. 1999. Toxicity of lead-contaminated sediment to mallards. *Archives of Environmental Contamination and Toxicology*, **36,** 323-333.

Helander, B., Olsson, A., Bignert, A., Asplund, L. & Litzén, K. 2002. The role of DDE, PCB, coplanar PCB and eggshell parameters for reproduction in the white-tailed sea eagle (*Haliaeetus albicilla*) in Sweden. *Ambio*, **31,** 386-403.

Helberg, M., Bustnes, J. O., Erikstad, K. E., Kristiansen, K. O. & Skaare, J. U. 2005. Relationships between reproductive performance and organochlorine contaminants in great black-backed gulls (*Larus marinus*). *Environmental Pollution*, **134,** 475-483.

Hernández, L. M., González, M. J. & Fernández, M. A. 1988. Organochlorines and metals in Spanish Imperial Eagle eggs, 1986-1987. *Environmental Conservation*, **15,** 363-364.

Herzke, D., Berger, U., Kallenborn, R., Nygård, T. & Vetter, W. 2005. Brominated flame retardants and other organobromines in Norwegian predatory bird eggs. *Chemosphere*, **61,** 441-449.

Hewett, T. 2003. Clean Scene: a report detailing coastal environmental improvement schemes and projects in the Solent region. (Ed. by Solent Forum). Winchester.

Hickey, J. J. & Anderson, D. W. 1968. Chlorinated hydrocarbons and eggshell changes in raptorial and fish-eating birds. *Science*, **162,** 271-273.

Hill, C. H. 1974a. Influence of high levels of minerals on the susceptibility of chicks to *Salmonella gallinarum*. *Journal of Nutrition*, **104,** 1221-1226.

Hill, E. F. & Camardese, M. B. 1986. Lethal dietary toxicities of environmental contaminants and pesticides to Coturnix. In: *US Fish and Wildlife Service Technical Report 2*: US Fish and Wildlife Service.

Hill, E. F., Heath, R. G., Spann, J. W. & Williams, J. D. 1975. Lethal dietary toxicities of environmental pollutants to birds. Washington, DC: US Department of Interior, Fish and Wildlife Service.

Hipfner, J. M. & Gaston, A. J. 1999. The relationship between egg size and posthatching development in the thick-billed murre. *Ecology*, **80,** 1289-1297.

Hipfner, J. M., Gaston, A. J., Herzberg, G. R., Brosnan, J. T. & Storey, A. E. 2003. Egg composition in relation to female age and relaying: Constraints on egg production in Thick-billed Murres (*Uria lomvia*). *The Auk*, **120,** 645-657.

Hoffman, D. J. 2002. Role of selenium toxicity and oxidative stress in aquatic birds. *Aquatic Toxicology*, **57,** 11-26.

Hoffman, D. J., Franson, J. C., Pattee, O. H., Bunck, C. M. & Anderson, A. 1985. Survival, growth and accumulation of ingested lead in nestling American kestrels (*Falco sparverius*). *Archive of Environmental Contamination and Toxicology*, **14,** 89-94.

Hoffman, D. J. & Heinz, G. H. 1988. Embryotoxic and teratogenic effects of selenium in the diet of mallards. *Journal of Toxicology and Environmental Health*, **24,** 477-490.

Hoffman, D. J., Heinz, G. H., LeCaptain, L. J., Bunck, C. M. & Green, D. E. 1991. Subchronic hepatoxicity of selenomethionine in mallard ducks. *Journal of Toxicology and Environmental Health*, **32,** 449-464.

Hoffman, D. J., Sanderson, C. J., LeCaptain, L. J., Cromartie, E. & Pendleton, G. W. 1992. Interactive effects of arsenate, selenium, and dietary protein on survival, growth, and physiology in mallard ducklings. *Archive of Environmental Contamination and Toxicology*, **22,** 55-62.

Hoffman, R. D. & Curnow, R. D. 1979. Mercury in herons, egrets, and their foods. *Journal of Wildlife Management*, **43,** 85-93.

Holcman, A. & Stibilj, V. 1997. Arsenic residues in eggs from laying hens fed with a diet containing arsenic (III) oxide. *Archive of Environmental Contamination and Toxicology*, **32,** 407-410.

Holz, R. & Starke, W. 1984. Biometrie, Geschlechtsdimorphismus und Eimasse mecklenburgischer Lachmöwen (*Larus ridibundus*). *Beiträge zur Vogelkunde Jena*, **30,** 297-304.

Honda, K., Yamamoto, Y., Hidaka, H. & Tatsukawa, R. 1986. Heavy metal accumulations in Adelie penguin, *Pygoscelis adeliae*, and their variations with the reproductive process. *Memoirs. National Institute of Polar Research (Japan)*, **Special Issue, 40,** 443-453.

Horn, H. S. 1968. The adaptive significance of colonial nesting in the Brewer's Blackbird (*Euphagus cyanocephalus*). *Ecology*, **49,** 682-694.

Hothem, R. L. & Welsh, D. 1994. Contaminants in eggs of aquatic birds from the grasslands of central California. *Archive of Environmental Contamination and Toxicology*, **27,** 180-185.

Houde, A. E. 1983. Nest density, habitat choice, and predation in a Common Tern colony. *Colonial Waterbirds*, **6,** 178-184.

Houghton, J. T., Callander, B. A. & Varney, S. K. 1992. *Climate Change 1992*. Cambridge, UK: Cambridge University Press.

Houston, D. C., Donnan, D., Jones, P., Hamilton, I. & Osbourne, D. 1995. Changes in the muscle condition of female Zebra finches *Poephila guttata* during egg laying and the role of protein storage in bird skeletal muscle. *Ibis*, **137,** 322-328.

Houston, D. C., Jones, P. J. & Sibley, R. M. 1983. The effect of female body condition on egg laying in Lesser black-backed Gulls *Larus fuscus*. *Journal of Zoology*, **200,** 509-520.

Howarth, D. M., Hulbert, A. J. & Horning, D. 1981. A comparative study of heavy metal accumulation in tissues of the crested tern, *Sterna bergii*, breeding near industrialised and non-industrialised areas. *Australian Wildlife Research*, **8,** 665-672.

Howell, G. N., O'Connor, M. J., Bond, A. M., Hudson, H. A., Hanna, P. J. & Strother, S. 1986. Methylmercury generation in seawater by transmethylation reactions of organo-lead and -tin compounds with inorganic mercury as monitored by multinuclear magnetic resonance and electrochemical techniques. *Australian Journal of Chemistry*, **39,** 1167-1175.

Hoyt, D. F. 1979. Practical methods of estimating volume and fresh weight of birds eggs. *The Auk*, **96,** 73-77.

HSDB. 2008a. Hazardous Substances Data Bank: selenium compounds. Toxicology Data Network; http://toxnet.nlm.nih.gov/.

HSDB. 2008b. Hazardous Substances Data Bank: iron compounds. Toxicology Data Network; http://toxnet.nlm.nih.gov/.

HSDB. 2008c. Hazardous Substances Data Bank: vanadium compounds. Toxicology Data Network; http://toxnet.nlm.nih.gov/.

HSDB. 2009a. Hazardous Substances Data Bank: lead compounds. Toxicology Data Network; http://toxnet.nlm.nih.gov/.

HSDB. 2009b. Hazardous Substances Data Bank: zinc compounds. Toxicology Data Network; http://toxnet.nlm.nih.gov/. In: *Toxicology Data Network; http://toxnet.nlm.nih.gov/.*

HSDB. 2009c. Hazardous Substances Date Bank: arsenic compounds. Toxicology Data Network; http://toxnet.nlm.nih.gov/.

HSDB. 2009d. Hazardous Substances Data Bank: cobalt compounds. Toxicology Data Network; http://toxnet.nlm.nih.gov/. In: *Toxicology Data Network; http://toxnet.nlm.nih.gov/.*

HSDB. 2009e. Hazardous Substances Data Bank: manganese compounds. Toxicology Data Network; http://toxnet.nlm.nih.gov/.

Hudson-Edwards, K. A., Macklin, M. G., Curtis, C. D. & Vaughan, D. J. 1997. Historic metal mining inputs to Tees river sediment. *Science of the Total Environment*, **194/195,** 437-445.

Hudson-Edwards, K. A., Macklin, M. G. & Taylor, M. P. 1999. 2000 years of sediment-borne heavy metal storage in the Yorkshire Ouse basin, NE England, UK. *Hydrological Processes*, **13,** 1087-1102.

Hudson, P. J. 1982. Nest site characteristics and breeding success in the razorbill *Alca torda*. *Ibis*, **124,** 355-359.

Hudson, R. H., Tucker, R. K. & Haegele, M. A. 1984. Handbook of toxicity of pesticides to wildlife. In: *US Fish and Wildlife Service Resource Publication 153*: US Fish and Wildlife Service.

Huebers, H. A. 1991. Iron. In: *Metals and Their Compounds in the Environment: Occurrence, Analysis and Biological Relevance, Part II.* (Ed. by Merian, E.), pp. 945-958. Weinheim, Germany: VCH Verlagsgesellschaft.

Hughes, M. F. & Kenyon, E. M. 1998. Dose-dependent effects on the disposition of monomethylarsonic acid and dimethylarsinic acid in the mouse after intravenous administration. *Journal of Toxicology and Environmental Health*, **53,** 101-118.

Hughes, M. F., Menache, M. & Thompson, D. J. 1994. Dose-dependent disposition of sodium arsenate in mice following acute oral exposure. *Fundamental and Applied Toxicology*, **22,** 80-89.

Hulse, M., Mahoney, J. S., Schroder, G. D., Hacker, C. S. & Pier, S. M. 1980. Environmentally acquired lead, cadmium, and manganese in the cattle egret, *Bubulcus ibis*, and the laughing gull, *Larus atricilla*. *Archives of Environmental Contamination and Toxicology*, **9,** 65-78.

Humphreys, J. & May, V. J. 2005. *The Ecology of Poole Harbour*. London: Elsevier.

Hunt, G. L., Jr. & Hunt, M. W. 1975. Reproductive ecology of the Western Gull: the importance of nest spacing. *Auk*, **92,** 270-279.

Hussein, A. S., Cantor, A. H. & Johnson, T. H. 1988. Use of high levels of dietary aluminium and zinc for inducing pauses in egg production of Japanese quail. *Poultry Science*, **67,** 1157-1165.

Hutson, G. D. 1977. Agnostic display and spacing in the black-headed gull, *Larus ridibundus*. *Animal Behaviour*, **25,** 765-773.

Hutton, M. 1981. Accumulation of heavy metals and selenium in three seabird species from the United Kingdom. *Environmental Pollution, Series A*, **26,** 129-145.

Ickes, S. K., Belant, J. L. & Dolbeer, R. A. 1998. Nest disturbance techniques to control nesting gulls. *Wildlife Society Bulletin*, **26,** 269-273.

Ikemoto, T., Kunito, T., Tanabe, S., Tsurumi, M., Sato, F. & Oka, N. 2005. Non-destructive monitoring of trace element levels in short-tailed albatrosses (*Phoebastria albatrus*) and black-

footed albatrosses (*Phoebastria nigripes*) from Torishima Island, Japan using eggs and blood. *Marine Pollution Bulletin*, **51,** 889-895.

Information Britain. 2009. Information Britain Town Guide. http://www.information-britain.co.uk/.

Ingersoll, R. T., Montgomery Jr., E. B. & Aposhian, H. V. 1995. Central system toxicity of manganese. *Fundamental and Applied Toxicology*, **27,** 106-113.

IPIECA. 2003. Case Study: BP in Dorset, southern England. In: *The Oil Industry: Operating in Sensitive Environments*. London: International Petroleum Industry Environmental Conservation Association.

Isakson, J., Persson, T. A. & Selin Lindgren, E. 2001. Identification and assessment of ship emissions and their effects in the harbour of Göteborg, Sweden. *Atmospheric Environment*, **35,** 3659-3666.

Ixer, R. A., Stanley, C. J. & Vaughan, D. J. 1979. Cobalt- nickel- and iron-bearing sulpharsenides from the north of England. *Mineralogical Magazine*, **43,** 389-395.

Jackson, M. J. 1989. Physiology of zinc: general aspects. In: *Zinc in Human Biology* (Ed. by Mills, C. F.), pp. 1-11. London: Springer-Verlag.

Jackson, N. 1977. The effect of dietary copper sulphate on laying performance, nutrient intake and tissue copper and iron levels of the mature, laying, domestic fowl. *British Journal of Nutrition*, **38,** 93-100.

Jackson, N., Stevenson, M. H. & Kirkpatrik, G. M. 1979. Effects of the protracted feeding of copper sulphate-supplemented diets to laying, domestic fowl on egg production and on specific tissues, with special reference to mineral content. *British Journal of Nutrition*, **42,** 253-266.

Janssens, E., Dauwe, T., Bervoets, L. & Eens, M. 2001. Heavy metals and selenium in feathers of great tits (*Parus major*) along a pollution gradient. *Environmental Toxicology and Chemistry*, **20,** 2815-2820.

Janssens, E., Dauwe, T., Bervoets, L. & Eens, M. 2002. Inter- and intraclutch variability in heavy metals in feathers of great tit nestlings (*Parus major*) along a pollution gradient. *Archives of Environmental Contamination and Toxicology*, **43,** 323-329.

Jaspers, V., Covaci, A., Maervoet, J., Dauwe, T., Voorspoels, S., Schepens, P. & Eens, M. 2005. Brominated flame retardants and organochlorine pollutants in eggs of little owls (*Athene noctua*) from Belgium. *Environmental Pollution*, **136,** 81-88.

JNCC. 1999. Information Sheet on Ramsar Wetlands (RIS): Poole Harbour. Peterborough: Joint Nature Conservation Committee.

Johnson, C. A., Moench, H., Wersin, P., Kugler, P. & Wenger, P. 2005. Solubility of antimony and other elements in samples taken from shooting ranges. *Journal of Environmental Quality*, **34,** 248-254.

Johnson, C. M. 1976. Selenium in the environment. *Research Reviews*, **62,** 101-130.

Johnson, K. L. & Younger, P. L. 2002. Hydrogeological and geochemical consequences of the abandonment of Frazer's Grove carbonate hosted Pb/Zn fluorspar mine, North Pennines, UK. *Geological Society, London, Special Publications*, **198,** 347-363.

Johnson, K. L. & Younger, P. L. 2005. Rapid manganese removal from mine waters using an aerated packed-bed bioreactor *Journal of Environmental Quality*, **34,** 987-993.

Karlsson, O. & Lilja, C. 2008. Eggshell structure, mode of development and growth rate in birds *Zoology*, **111,** 494-502.

Karlsson, T. 2005. Egg size in Black-Headed Gulls *Larus ridibundus*. *Ornis Svecica*, **15,** 46-47.

Kashani, A. B., Samie, H., Emerick, R. J. & Carlson, C. W. 1986. Effect of copper on three levels of sulphur containing amino acids in diets for turkeys. *Poultry Science*, **65,** 1754-1759.

Kiekens, L. 1995. Zinc. In: *Heavy Metals in Soils, Second Edition* (Ed. by Alloway, B. J.). London: Blackie Academic and Professional.

Kilpi, M., Hillstrom, L. & Lindstrom, K. 2008. Egg-size variation and reproductive success in the Herring Gull *Larus argentatus*: adaptive or constrained size of the last egg? *Ibis*, **138,** 212-217.

Kim, S.-Y. & Monaghan, P. 2005. Interacting effects of nest shelter and breeder quality on behaviour and breeding performance of herring gulls. *Animal Behaviour*, **69,** 301-306.

King, K. A., Lefever, C. A. & Mulhern, B. M. 1983. Organochlorine and metal residues in royal terns nesting on the central Texas coast. *Journal of Field Ornithology*, **54,** 295-303.

Kirk-Othmer. 1995. *Kirk-Othmer Encyclopaedia of Chemical Technology, Vol. 1, 4th edition*. New York: John Wiley & Sons.

Kirkman, F. 1937. *Bird Behaviour*. London: T. Nelson & Sons, Ltd.

Klumpp, D. W. & Peterson, P. J. 1979. Arsenic and other trace elements in the waters and organisms of an estuary in SW England. *Environmental Pollution*, **19,** 11-20.

Krause, J. & Ruxton, G. D. 2002. *Living in Groups*. Oxford: Oxford University Press.

Kruuk, H. 1964. Predators and anti-predator behaviours of the Black-headed Gull (*Larus ridibundus* L.). *Behaviour*, **Suppl. 11,** 1-130.

Kubena, L. F., Phillips, T. D., Witzel, D. A. & Heidelbaugh, N. D. 1980. Influence of various levels of vanadium on female laying strain chickens. *Poultry Science*, **59,** 1628.

Kuchida, K., Fukaya, M., Miyoshi, S., Suzuki, M. & Tsuruta, S. 1999. Nondestructive prediction method for yolk:albumen ratio in chicken eggs by computer image analysis. *Poultry Science*, **78,** 909-913.

Kullberg, C., Jakobsson, S., Kaby, U. & Lind, J. 2005. Impaired flight ability prior to egg-laying: a cost of being a capital breeder. *Functional Ecology*, **19,** 98-101.

Labare, M. P., Butkus, M. A., Riegner, D., Schommer, N. & Atkinson, J. 2004. Evaluation of lead movement from the abiotic to the biotic at a small arms firing range. *Environmental Geology*, **46,** 750-754.

Lack, D. 1967. Interrelationships in breeding adaptations as shown by marine birds. *Proceedings of the International Ornithological Congress*, **14,** 3-42.

Laden, F., Neas, L. M., Dockery, D. W. & Schwartz, J. 2000. Association of fine particulate matter from different sources with daily mortality in six US cities. *Environmental Health Perspectives*, **108,** 941-947.

Lagerwerff, J. V. 1967. Heavy-metal contamination of soils. In: *Agriculture and the quality of our environment* (Ed. by Brady, N. C.), pp. 343-364. Norwood, Massachussetts: Plimpton Press.

Lam, J. C. W., Tanabe, S., Lam, M. H. W. & Lam, P. K. S. 2005. Risk to breeding success of waterbirds by contaminants in Hong Kong: evidence from trace elements in eggs. *Environmental Pollution*, **135,** 481-490.

Langston, W. J., Chesman, B. S., Burt, G. R., Hawkins, S. J., Readman, J. & Worsfold, P. 2003. Site characterisation of the South West European marine sites: Poole Harbour Special Protection Area. In: *Marine Biological Association Occasional publication No. 12*. Plymouth, UK: Marine Biological Association.

Laskey, J. W., Rehnberg, G. L., Hein, J. F. & Carter, S. D. 1982. Effects of chronic manganese (Mg3O4) exposure on selected reproductive parameters in rats. *Journal of Toxicology and Environmental Health*, **9,** 677-687.

Law, R. J., Dawes, V. J., Woodhead, R. J. & Matthiessen, P. 1997. Polycyclic aromatic hydrocarbons (PAH) in seawater around England and Wales. *Marine Pollution Bulletin*, **34,** 306-322.

Leach, R. M., Wang, K. W.-L. & Baker, D. E. 1979. Cadmium and the food chain: the effect of dietary cadmium on tissue composition in chicks and laying hens. *Journal of Nutrition*, **109,** 437-443.

LeBlanc, P. J. & Jackson, A. L. 1973. Arsenic in marine fish and invertebrates *Marine Pollution Bulletin*, **4,** 88-90.

Lee, K. 1989. The impact of hushing on a channel and flood plain morphology: a case study of the Hudeshope Beck, Upper Teesdale. University of Newcastle upon Tyne, BSc thesis.

Legret, M. & Pagotto, C. 1999. Evaluation of pollutant loadings in the runoff waters from a major rural highway. *Science of the Total Environment*, **235,** 143-150.

Lemmetyinen, R., Rantamaki, P. & Karlin, A. 1982. Levels of DDT and PCBs in different stages of life cycle of the arctic tern (*Sterna paradisaea*) and the herring gull (*Larus argentatus*). *Chemosphere*, **11,** 1059-1068.

Lessells, C. M., Dingemanse, N. J. & Both, C. 2002. Egg weights, egg component weights, and laying gaps in Great Tits (*Parus major*) in relation to ambient temperature. *The Auk*, **119,** 1091-1103.

Lewin, J., Davies, B. E. & Wolfenden, P. J. 1977. Interactions between channel change and historic mining sediments In: *River channel changes* (Ed. by Gregory, K. J.), pp. 1009-1027. Chichester: John Wiley and Sons.

Lewin, J. & Macklin, M. G. 1987. Metal mining and floodplain sedimentation in Britain. In: *International Geomorphology 1986 Part 1* (Ed. by Gardiner, V.), pp. 1009-1027. Chichester: John Wiley and Sons.

Lewis, S. A., Becker, P. H. & Furness, R. W. 1993. Mercury levels in eggs, internal tissues and feathers of herring gulls *Larus argentatus* from the German Wadden Sea coast. *Environmental Pollution*, **80,** 293-299.

Lewis, S. A. & Furness, R. W. 1991. Mercury accumulation and excretion in laboratory reared Black-headed Gull (*Larus ridibundus*) chicks. *Archives of Environmental Contamination and Toxicology*, **21,** 316-320.

Lide, D. R. 2008. *CRC Handbook of Chemistry and Physics, 89th edition*. London: CRC Press.

Lin, Z., Comet, B., Qvarfort, U. & Herbert, R. 1995. The chemical and mineralogical behaviour of Pb in shooting range soils from central Sweden. *Environmental Pollution*, **89,** 303-309.

Llobet, J. M. & Domingo, J. L. 1984. Acute toxicity of vanadium compounds in rats and mice. *Toxicology Letters*, **23,** 227-231.

Lloyd, C., Tasker, M. L. & Partridge, K. 1991. *The Status of Seabirds in Britain and Ireland*. London: T & AD Poyser.

Lock, J. W., Thompson, D. R., Furness, R. W. & Bartle, J. A. 1992. Metal concentrations in seabirds to the New Zealand region. *Environmental Pollution*, **75,** 289-300.

Lu, J. & Combs Jr., G. E. 1988. Effects of excess dietary zinc on pancreatic exocrine function in the chick. *Journal of Nutrition*, **118,** 681-689.

Lukowski, A. B. 1978. The content of organochlorine insecticides in the eggs and tissues of young individuals of the Great Crested Grebe (*Podiceps cristatus*) and of the Coot (*Fulica atra* L.) from the Mausaurian lakes. *Ekologia Polska*, **26,** 467-478.

Lundberg, C.-A. & Väisänen, R. A. 1979. Selective correlation of egg size with chick mortality in the Black-headed Gull (*Larus ridibundus*). *Condor*, **81,** 146-156.

Lundholm, C. E. 1987. Methyl mercury decreases the eggshell thickness and inhibits the Ca2+ uptake in a homogenate of the eggshell gland mucosa and its subcellular fractions from the domestic fowl. *Pharmacology and Toxicology*, **60,** 385-388.

Lundholm, C. E. 1995. Effects of methyl mercury at different dose regimes on eggshell formation and some biochemical characteristics of the eggshell gland mucosa of the domestic fowl. *Comparative Biochemistry and Physiology*, **110C,** 23-28.

Macklin, M. G. 1985. Floodplain sedimentation in the Upper Axe valley, Mendip, England. *Transactions of the Institute of British Geographers*, **NS 10,** 235-244.

Macklin, M. G. 1992. Metal contaminated soils and sediment: a geographical perspective. In: *Managing the human impact on the natural environment: patterns and processes* (Ed. by Newson, M. D.), pp. 174-195. London: Belhaven Press.

Macklin, M. G., Rumsby, B. T. & Newson, M. D. 1992. Historical floods and vertical accretion of fine-grained alluvium in the Lower Tyne valley, North East England. In: *Dynamics of gravel-bed rivers* (Ed. by Billi, P., Hey, R. D., Thorne, C. R. & Tacconi, P.), pp. 573-589. Chichester: John Wiley and Sons.

Maeda, S., Inoue, R., Kozono, T., Tokuda, T., Ohki, A. & Takeshita, T. 1990. Arsenic metabolism in a freshwater food chain. *Chemosphere*, **20,** 101-108.

Maedgen, J. L., Hacker, C. S., Schroder, G. D. & Weir, F. W. 1982. Bioaccumulation of lead and cadmium in the royal tern and sandwich tern. *Archive of Environmental Contamination and Toxicology*, **11,** 99-102.

Magat, W. & Sell, J. L. 1979. Distribution of mercury and selenium in egg components and egg-white proteins. *Proceedings of the Society for Experimental Biology and Medicine*, **161,** 458-461.

Makepeace, D. K., Smith, D. W. & Stanley, S. J. 1995. Urban stormwater quality: summary of contaminant data. *Critical Reviews in Environmental Science and Technology*, **25,** 93-139.

Malling Olsen, K. & Larsson, H. 2004. *Gulls of Europe, Asia and North America*. London: Christopher Helm, A&C Black Publishers Ltd.

Manning, W. 1959. *The future of non-ferrous mining in GB and Ireland*. London: Institution of Mining and Metallurgy.

Martin, T. E. & Roper, J. J. 1988. Nest predation and nest-site selection of a western population of the Hermit Thrush. *Condor*, **90,** 51-57.

Martin, W. E. & Nickerson, P. R. 1973. Mercury, lead, cadmium, and arsenic residues in starlings - 1971. *Pesticides Monitoring Journal*, **7,** 67-72.

Mason, R. P., Laporte, J. M. & Andres, S. 2000. Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish. *Archive of Environmental Contamination and Toxicology*, **38,** 283-297.

May, T. W. & Wiedmeyer, R. H. 1998. A table of polyatomic interferences in ICP-MS. *Atomic Spectroscopy*, **19,** 150-155.

Mayack, L. A., Bush, P. B., Fletcher, O. J., Page, R. K. & Fendley, T. T. 1981. Tissue residues of dietary cadmium in wood ducks. *Archives of Environmental Contamination and Toxicology*, **10,** 637-645.

McGrath, S. P. 1995. Nickel. In: *Heavy Metals in Soils, Second Edition* (Ed. by Alloway, B. J.). London: Blackie Academic and Professional.

McNamara, J. N. & Houston, A. I. 1996. State-dependant life histories. *Nature*, **380,** 215-221.

Meathrel, C. E. & Ryder, J. P. 1987. Intraclutch variation in the size, mass and composition of ring-billed gull eggs. *The Condor*, **89,** 364-368.

Mergler, D. 1986. Manganese: the controversial metal. At what levels can deleterious effects occur? *Canadian Journal of Neurological Science*, **23,** 93-94.

Mermoz, M. E. & Reboreda, J. C. 1998. Nesting success in Brown-and yellow Marshbirds: effects of timing, nest site, and brood parasitism. *The Auk*, **115,** 871-878.

Met Office UK. 2009. Regional mapped climate averages 1971-2000. http://www.metoffice.gov.uk/climate/uk/averages/.

Migliorini, M., Pigino, G., Bianchi, N., Bernini, F. & Leonzio, C. 2003. The effects of metal contamination on the soil arthropod community of a shooting range. *Environmental Pollution*, **129,** 331-340.

Miles, A. K., Pendleton, G. W. & Soares, J. H., Jr. 1993. Effects of dietary aluminium, calcium and phosphorus on egg and bone of European starlings. *Archives of Environmental Contamination and Toxicology*, **24,** 206-212.

Mineau, P. 1982. Levels of major organochlorine contaminants in sequentially-laid herring gull eggs. *Chemosphere*, **11,** 679-685.

Mitchell, P. I., Newton, S. F., Ratcliffe, N. & Dunn, T. E. 2004. *Seabird Populations of Britain and Ireland*. London: T. & A.D. Poyser.

Miyazaki, M. 1996. Vegetation cover, kleptoparasitism by diurnal gulls, and timing of arrival of nocturnal Rhinocerous Auklets. *The Auk*, **113,** 698-702.

Moksnes, K. 1983. Selenium deposition in tissues and eggs of laying hens given surplus of selenium as selenomethionine. *Acta Veterinaria Scandanavica*, **24,** 34-44.

Monaghan, P. & Nager, R. G. 1997. Why don't birds lay more eggs? *Trends in Ecology and Evolution*, **12,** 270-274.

Monaghan, P., Nager, R. G. & Houston, D. C. 1998. The price of eggs: increased investment in egg production reduces the offspring rearing capacity of parents. *Proceedings of the Royal Society London: Biological Sciences*, **265,** 1731-1735.

Montevecchi, W. A. 1978. Nest site selection and its survival value among laughing gulls. *Behavioural Ecology and Sociobiology*, **4,** 143-161.

Mora, M. A. 2003. Heavy metals and metalloids in egg contents and eggshells of passerine birds from Arizona. *Environmental Pollution*, **125,** 393-400.

Mora, M. A., Montoya, A. B., Lee, M. C., Macías-Duarte, A., Rodríguez-Salazarb, R., Juergens, P. W. & Lafón-Terrazas, A. 2008. Persistent environmental pollutants in eggs of Aplomado falcons from Northern Chihuahua, Mexico, and South Texas, USA *Environment International*, **34,** 44-50.

Morel, F. M. M., Westall, J. C., O'Melia, C. R. & Morgan, J. J. 1975. Fate of trace metals in Los Angeles County waste water discharge. *Environmental Science and Technology*, **9,** 756-761.

Moreng, R. E., Balnave, D. & Zhang, D. 1992. Dietary zinc methionine effect on eggshell quality of hens drinking saline water. *Poultry Science*, **71,** 1163-1167.

Morera, M., Sanpera, C., Crespo, S., Jover, L. & Ruiz, X. 1997. Inter- and intraclutch variability in heavy metals and selenium levels in Audouin's gull eggs from the Ebro Delta, Spain. *Archives of Environmental Contamination and Toxicology*, **33,** 71-75.

Moriyama, K., Mori, T., Arayashiki, H., Saito, A. & Chino, M. 1989. The amount of heavy metals derived from domestic wastewater. *Water Science and Technology*, **21,** 1913-1916.

Morris, D. W. 1989. Density-dependant habitat selection: testing the theory with fitness data. *Evolutionary Ecology*, **3,** 80-94.

Morrissey, C. A., Albert, C. A., Dods, P. L., Cullen, W. R., Lai, V. W.-M. & Elliott, J. E. 2007. Arsenic accumulation in bark beetles and forest birds occupying mountain pine beetle infested stands treated with monosodium methanearsonate. *Environmental Science and Technology*, **41,** 1494-1500.

Moss, R., Watson, A., Rothery, P. & Glennie, W. W. 1981. Clutch size, egg size, hatch weight and laying date in relation to early mortality in red grouse *Lagopus lagopus scoticus* chicks. *Ibis*, **123,** 450-462.

Mott MacDonald. 2010. Hamble Jetty, UK. http://www.maritime.mottmac.com/projects/?mode=type&id=169727: Mott MacDonald Group Ltd.

Muirhead, S. J. & Furness, R. W. 1988. Heavy metal concentrations in the tissues of seabirds from Gough Island, South Atlantic Ocean. *Marine Pollution Bulletin*, **19,** 278-283.

Muralidharan, S., Jayakumar, R. & Vishnu, G. 2004. Heavy metals in feathers of six species of birds in the District Nilgris, India. *Bulletin of Environmental Contamination and Toxicology*, **73,** 285-291.

Murdoch, N. & Randall, D. 2001. Poole Harbour Water Quality Model (Nitrogen). pp. 22: Environment Agency.

Murton, R. K., Osborn, D. & Ward, P. 1978. Are heavy metals pollutants in Atlantic seabirds? *Ibis*, **120,** 106-107.

Nager, R. G., Monaghan, P. & Houston, D. C. 2000. Within-clutch trade-offs between the number and quality of eggs: experimental manipulations in gulls. *Ecology*, **81,** 1339-1350.

Nagorski, S. A., McKinnon, T. E. & Moore, J. N. 2003. Seasonal and storm-scale variations in heavy metal concentrations of two mining-contaminated streams, Montana, USA. *Journal de Physique IV*, **107,** 909-912.

NAS. 1973. Manganese. (Ed. by United States National Academy of Sciences (NAS)). Washington, DC: University Park Press.

NAS. 1975. Nickel: medical and biologic effects of environmental pollutants. (Ed. by United States National Academy of Sciences (NAS)). Washington, DC: University Park Press.

NAS. 1976. Selenium: medical and biologic effects of environmental pollutants. (Ed. by United States National Academy of Sciences (NAS)). Washington, DC: University Park Press.

NAS. 1977a. Arsenic. (Ed. by United States National Academy of Sciences (NAS)). Washington, DC: University Park Press.

NAS. 1977b. Copper. (Ed. by United States National Academy of Sciences (NAS)). Washington, DC: University Park Press.

NAS. 1979. Zinc. (Ed. by United States National Academy of Sciences (NAS)). Baltimore: University Park Press.

NAS. 1980. *Mineral tolerance of domestic animals*. Washington, DC: National Research Council, Subcommittee on Mineral Toxicity in Animals.

Natural England. 2009. England's Geology: Hampshire. http://www.naturalengland.org.uk/ourwork/conservation/geodiversity/englands/counties/area_ID16.aspx.

NC DPPEA. 1998. Factsheet: Lead contamination in soils at military small arms firing ranges. North Carolina Division of Pollution Prevention and Environmental Assistance.

Neal, C. & Davies, H. 2003. Water quality fluxes for eastern UK rivers entering the North Sea: a summary of information from the Land-Ocean Interaction Study (LOIS). *Science of the Total Environment*, **314/316,** 821-882.

Needleman, H. L., Schell, A., Bellinger, D., Leviton, A. & Allred, E. N. 1990. The long-term effects of exposure to lead in childhood, an 11 year follow-up report. *New England Journal of Medicine*, **322,** 83-88.

Nelson, B. 1978. *The Gannet*. Vermillion, South Dakota: Buteo Books.

Nettleship, D. N. 1972. Breeding success of the common puffin (*Fratercula Arctica* L.) on different habitats at Great Island, Newfoundland. *Ecological Monographs*, **42,** 239-266.

New Forest District Council. 2004a. New Forest District Council Management Plan. Zone 6: Lymington River Estuary.

New Forest District Council. 2004b. Minutes of a meeting of the New Forest Environmental Protection Liaison Committee held at Appletree Court, Lyndhurst on 11 June 2004. http://www.newmiltontowncouncil.gov.uk/committeedocs/nfeplc/NFR17979.pdf: New Forest Environmental Protection Liaison Committee.

New Forest District Council. 2009. Proposed intertidal mud-flat recharge, Lymington River. http://www.newforest.gov.uk/committeedocs/cab/CDR05176.pdf.

New Forest District Council. 2010. Farming facts in the New Forest National Park. http://www.newforestnpa.gov.uk/index/lookingafter/la-landscape/la-farmlandinnewforest/farming_facts.htm.

New Forest NPA. 2008. New Forest National Park Plan: Topic Paper 1 - Biodiversity, Geology and Landscape. New Forest National Park Association, www.newforestnpa.gov.uk/biodiversity_topic_paper.pdf.

Newton, I. 1973. Egg breakage and breeding failure in British Merlins. *Bird Study*, **20,** 241-244.

Newton, I., Meek, E. R. & Little, B. 1978. Breeding ecology of the Merlin in Northumberland. British Birds. *British Birds*, **7,** 376-398.

Newton, I., Robson, J. E. & Yalden, D. W. 1983. Decline of the Merlin in the Peak District. *Bird Study*, **18,** 225-234.

Nice, M. M. 1962. Development of behavior in precocial birds. *Linnaean Society of New York; Transactions*, **8,** 1-211.

Nisbet, I. C. T. 1978. Dependence of fledging success on egg size, parental performance and egg composition among common and roseate terns, *Sterna hirundo* and *S. dougallii*. *Ibis*, **120,** 207-215.

Nisbet, I. C. T. 1982. Eggshell characteristics and organochlorine residues in common terns: variation with egg sequence. *Colonial Waterbirds*, **5,** 139-143.

NOAA. 2007. Pollution: stressors from marina and boating activities. www.coastalmanagement.noaa.gov/initiatives/pollution_sup.html: Office of Ocean and Coastal Resource Management, National Oceanic and Atmospheric Administration, US Department of Commerce.

North Pennines AONB Partnership. 2010. Geology and Landscape. North Pennines Area of Outstanding Natural Beauty and UNESCO European Geopark. www.northpennines.org.uk/index.cfm?articleid=713.

NRCC. 1978. Effects of arsenic in the Canadian environment, Publication No. NRCC 15391. pp. 349: Natural Resource Council Canada.

Nriagu, J. O. 1989. A global assessment of natural sources of atmospheric trace metals. *Nature*, **338,** 47-49.

Nriagu, J. O. & Coker, R. D. 1980. Trace metals in humic and fulvic acids from Lake Ontario sediments. *Environmental Science and Technology*, **14,** 443-446.

Nriagu, J. O. & Pacyna, J. M. 1988. Quantitative assessment of worldwide contamination of air, water, and soils by trace metals. *Nature*, **333,** 134-139.

NVSWC. 2005. Heavy metal pollution is more common than you think. Virginia, USA: Conservation Currents, Northern Virginia Soil and Water Conservation District.

Nybø, S., Staurnes, M. & Jerstad, K. 1997. Thinner eggshells of dipper (*Cinclus cinclus*) eggs from an acidified area compared to a non-acidified area in Norway. *Water, Air, and Soil Pollution*, **93,** 255-266.

O'Connor, R. J. 1975. An adaptation for early growth in tits, Parus *spp*. *Ibis*, **117,** 523-526.

Oh, S. H., Nakaue, H., Deagen, J. T., Whanger, P. D. & Arscott, G. H. 1979. Accumulation and depletion of zinc in chick tissue metallothioneins. *Journal of Nutrition*, **109,** 1720-1729.

OhioEPA. 1995. Fact Sheet No. 30: Pollution Prevention for Marinas. www.p2pays.org/ref/11/10419.htm: Ohio Environmental Protection Agency.

Ohlendorf, H. M. 1989. Bioaccumulation and effects of selenium in wildlife. In: *Selenium in Agriculture and the Environment, Special Publication 23* (Ed. by Jacobs, L. W.), pp. 133-177. Madison, Wisconsin: American Society of Agronomy and Soil Science Society of America.

Ohlendorf, H. M., Hoffman, D. J., Saiki, M. K. & Aldrich, T. W. 1986. Embryonic mortality and abnormalities of aquatic birds: apparent impacts of selenium from irrigation drainwater. *Science of the Total Environment*, **52,** 49-63.

Ohlendorf, H. M., Kilness, A. W., Simmons, J. L., Stroud, R. K., Hoffman, D. J. & Moore, J. F. 1988. Selenium toxosis in wild aquatic birds. *Journal of Toxicology and Environmental Health*, **24,** 67-92.

Ohlendorf, H. M., Marois, K. C., Lowe, R. W., Harvey, T. E. & Kelly, P. R. 1991. Trace elements and organochlorines in surf scoters from San Francisco Bay, 1985. *Environmental Monitoring and Assessment*, **18,** 105-122.

Ohnesorge, F. K. & Wilhelm, M. 1991. Zinc. In: *Metals and Their Compounds in the Environment: Occurrence, Analysis and Biological Relevance* (Ed. by Merian, E.). Weinhelm, Germany: VCH Verlagsgesellschaft.

Oladimeji, A. A., Qadri, S. U., Tam, G. K. H. & DeFreitas, A. S. W. 1979. Metabolism of inorganic arsenic to organoarsenicals in rainbow trout (*Salmo gairdneri*). *Ecotoxicology and Environmental Safety*, **3,** 394-400.

Ollason, J. C. & Dunnet, G. M. 1978. Age, experience and other factors affecting the breeding success of the fulmar, *Fulmarus glacialis*, in Orkney. *Journal of Animal Ecology*, **47,** 961-976.

Olsson, E. 1999. Plug the leaks: small spill workshop. Alaska: Oil Spill Recovery Institute.

Ormerod, S. J., Tyler, S. J. & Jüttner, I. 2000. Effects of point-source PCB contamination on breeding performance and post-fledgling survival in the dipper *Cinclus cinclus*. *Environmental Pollution*, **109,** 505-513.

Oro, D., Pradel, R. & Lebreton, J.-D. 1999. Food availability and nest predation influence life history traits in Audouin's Gull, *Larus audouinii*. *Oecologia*, **118,** 438-445.

Ort, J. F. & Latshaw, J. D. 1978. The toxic level of sodium selenite in the diet of laying chickens. *Journal of Nutrition*, **108,** 1114-1120.

Osborn, D., Every, W. J. & Bull, K. R. 1983. The toxicity of trialkyllead compounds to birds *Environmental Pollution*, **31,** 261-275.

Osborn, D., Harris, M. P. & Nicholson, J. K. 1979. Comparative tissue distribution of mercury, cadmium and zinc in three species of pelagic seabirds. *Comparative Biochemistry and Physiology*, **64,** 61-67.

Ousterhout, L. E. & Berg, L. R. 1981. Effects of diet composition on vanadium toxicity in laying hens. *Poultry Science*, **60,** 1152-1159.

Outridge, P. M. & Scheuhammer, A. M. 1993. Bioaccumulation and toxicology of nickel: implications for wild mammals and birds. *Environmental Reviews*, **1,** 172-197.

Pacyna, E. G., Pacyna, J. M., Fudala, J., Strzelecka-Jastrzab, E., Hlawiczka, S., Panasiuk, D., Nitter, S., Pregger, T., Pfeiffer, H. & Friedrich, R. 2007. Current and future emissions of selected heavy metals to the atmosphere from anthropogenic sources in Europe *Atmospheric Environment*, **41,** 8557-8566.

Page, A. L., Chang, A. C. & El-Amamy, M. 1987. Cadmium levels in soils and crops in the United States. In: *Lead, Mercury, Cadmium and Arsenic in the Environment* (Ed. by Hutchinson, T. C. & Meema, K. M.). California: John Wiley & Sons Ltd.

Pain, D. J. 1996. Lead in Waterfowl. In: *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations* (Ed. by Beyer, W. N., Heinz, G. H. & Redmon-Norwood, A. W.), pp. 251-264. Boca Raton, Florida: Lewis Publishers, CRC Press.

Pain, D. J., Amiard-Triquet, C. & Sylvestre, C. 1992. Tissue lead concentrations and shot ingestion in nine species of waterbirds from the Camargue (France). *Ecotoxicology and Environmental Safety*, **24,** 217-233.

Pain, D. J., Burneleau, G., Bevoux, C. & Wyatt, C. 1999. Levels of polychlorinated biphenyls, organochlorine pesticides, mercury and lead in relation to shell thickness in marsh harrier (*Circus aeruginosus*) eggs from Charente-Maritime, France. *Environmental Pollution*, **104,** 61-68.

Palafox, A. L. & Ho-A, E. 1980. Effect of zinc toxicity in laying white leghorn pullets and hens. *Poultry Science*, **59,** 2024-2028.

Paludan, K. 1951. Contributions to the breeding biology of *Larus argentatus* and *L. fuscus*. *Videnskabelige Meddelelser Dansk Naturhistorisk Forening*, **114,** 1-128.

Parker, R. D. R. & Sharma, R. P. 1978. Accumulation and depletion of vanadium in selected tissues of rats treated with vanadyl sulphate and sodium orthovanadate. *Journal of Environmental Pathology and Toxicology*, **2,** 235-245.

Parslow, J. L. F., Jefferies, D. J. & French, M. C. 1972. Ingested pollutants in puffins and their eggs. *Bird Study*, **19,** 18-33.

Parslow, J. L. F. & Jeffries, D. J. 1977. Gannets and toxic chemicals. *British Birds*, **70,** 366-372.

Parsons, J. 1970. Relationship between egg size and post-hatching mortality in the herring gull (*Larus argentatus*). *Nature*, **228,** 1221-1222.

Parsons, J. 1971. Cannibalism in Herring Gulls. *British Birds*, **64,** 528-537.

Parsons, J. 1972. Egg size, laying date and incubation period in the Herring Gull. *Ibis*, **114,** 536-541.

Parsons, J. 1975. Seasonal variation in breeding success of the Herring Gull: an experimental approach to pre-fledging success. *Journal of Animal Ecology*, **44,** 553-573.

Parsons, J. 1976a. Factors determining the number and size of eggs laid by the Herring Gull. *Condor*, **78,** 481-492.

Parsons, J. 1976b. Nesting density and breeding success in the Herring Gull *Larus argentatus*. *Ibis*, **118,** 537-546.

Partridge, L. 1978. Habitat selection. In: *Behavioural Ecology: and Evolutionary Approach* (Ed. by Krebs, J. R. & Davis, N. B.), pp. 351-376. Oxford: Blackwell Scientific.

Patin, S. 1999. *Environmental Impact of the Offshore Oil and Gas Industry*. New York: EcoMonitor Publishing.

Pattee, O. H. 1984. Eggshell thickness and reproduction in American kestrels exposed to chronic dietary lead. *Archive of Environmental Contamination and Toxicology*, **13,** 29-34.

Patterson, I. J. 1965. Timing and spacing of broods in the Black-headed Gull. *Ibis*, **107,** 433-459.

Paynter, R. 1949. Clutch size and the egg and chick mortality of Kent Island Herring Gulls. *Ecology*, **30,** 146-166.

Peakall, D. B. 1992. *Animal Biomarkers as Pollution Indicators*. London: Chapman & Hall.

Peakall, D. B. & Lincer, J. L. 1996. Do PCBs cause eggshell thinning? *Environmental Pollution*, **91,** 127-129.

Penrose, W. R. 1975. Biosynthesis of organic arsenic compounds in brown trout (*Salmo trutta*). *Journal of the Fisheries Research Board of Canada*, **32,** 2385-2390.

Perrins, C. M. 1970. The timing of birds' breeding seasons. *Ibis*, **112,** 242-255.

Perrins, C. M. 1996. Eggs, egg formation and the timing of breeding. *Ibis*, **138,** 2-15.

Pescatore, A. J. & Harter-Dennis, J. M. 1989. Effects of ferrous sulfate consumption on the performance of broiler chicks. *Poultry Science*, **68,** 1063-1067.

Peters, S. C. & Blum, J. D. 2003. The source and transport of arsenic in a bedrock aquifer, New Hampshire, USA *Applied Geochemistry*, **18,** 1773-1787.

Pettine, M., Camusso, M. & Martinotti, W. 1992. Dissolved and particulate transport of arsenic and chromium in the Po River (Italy). *Science of the Total Environment*, **119,** 253-280.

PHC. 2007. Poole Harbour - The Harbour Today: Leisure. www.phc.co.uk/Leisure.htm: Poole Harbour Commissioners (PHC).

Pierotti, R. 1982. Habitat selection and its effect on reproductive output in the herring gull in Newfoundland. *Ecology*, **63,** 854-868.

Pierotti, R. & Annett, C. A. 1990. Diet and reproductive output in seabirds. *BioScience*, **40,** 568-574.

Pilastro, A., Congiu, L., Tallandini, L. & Turchetto, M. 1993. The use of bird feathers for the monitoring of cadmium pollution. *Archives of Environmental Contamination and Toxicology*, **24,** 355-358.

Poole Tourism. 2009. Poole Tourism: Car Parking. http://www.pooletourism.com/go.php?structureID=pages&ref=I482EE3A303F93.

Potts, G. R. 1968. Success of eggs of shags on the Farne Islands, Northumberland, in relation to their content of dieldrin and pp'DDE. *Nature*, **217,** 1282-1284.

Potts, G. R., Coulson, J. C. & Deans, I. R. 1980. Population dynamics and breeding success of the shag *Phalacrocorax aristotelis* on the Farne Islands, Northumberland. *Journal of Animal Ecology*, **49,** 465-484.

Poupoulis, C. & Jensen, L. S. 1976. Effects of high dietary copper on gizzard integrity of the chick. *Poultry Science*, **55,** 113-121.

Preston, F. W. & Preston, E. J. 1953. Variation in the shapes of birds' eggs within a clutch. *Annals of Carnegie Museum*, **33,** 129-139.

Pritzl, M. C., Lie, Y. H., Kienholz, E. W. & Whiteman, C. E. 1974. The effect of dietary cadmium on development of young chickens. *Poultry Science*, **53,** 2026-2029.

Proctor, N. H., Hathaway, G. J. & Hughes, J. P. 2004. *Proctor and Hughes' Chemical Hazards of the Workplace*. New Jersey: John Wiley & Sons Inc.

Pugesek, B. H. 1981. Increased reproductive effort with age in the California gull (*Larus californicus*). *Science*, **212,** 822-823.

Rahn, H., Carey, C., Balmas, H., Bhatia, B. & Paganelli, C. 1977. Reduction of pore area of the avian eggshell as an adaptation to altitude. *The Proceedings of the National Academy of Sciences*, **74,** 3095-3098.

Ratcliffe, D. A. 1967. Decrease in eggshell weight in certain birds of prey. *Nature*, **215,** 208-210.

Ratcliffe, D. A. 1970. Changes attributable to pesticides in egg breakage frequency and eggshell thickness in some British birds. *Journal of Applied Ecology*, **7,** 67-115.

Ratcliffe, N., Furness, R. W. & Hamer, K. C. 1998. The interactive effects of age and food supply on the breeding ecology of great skuas. *Journal of Animal Ecology*, **67,** 853-862.

Rattner, B. A., McKernan, M. A., Eisenrech, K. M., Link, W. A., Olsen, G. H., Hoffman, D. J., Knowles, K. A. & McGowan, P. C. 2006. Toxicity and hazard of vanadium to mallard ducks (*Anas platyrhynchos*) and Canada geese (*Branta canadensis*). *Journal of Toxicology and Environmental Health, Part A*, **69,** 331-351.

Reid, W. V. & Boersma, P. D. 1990. Parental quality and selection on egg size in the Magellanic penguin. *Evolution*, **44,** 1780-1786.

Renzoni, A., Focardi, S., Fossi, C., Leonzio, C. & Mayol, J. 1986. Comparison between concentrations of mercury and other contaminants in eggs and tissues of Cory's shearwater *Calonectris diomedea* collected on Atlantic and Mediterranean islands. *Environmental Pollution, Series A*, **40,** 17-35.

Rhymer, J. M. 1988. The effect of egg size variability on thermoregulation of Mallard (*Anas platyrhynchos*) offspring and its implication for survival. *Oecologia*, **75,** 20-24.

Rice, D. C. 1996. Behavioural effects of lead: Commonalities between experimental and epidemiologic data. *Environmental Health Perspectives*, **104,** 337-351.

Richards, M. P. 1989. Recent developments in trace element metabolism and function: role of metallothionein in copper and zinc metabolism. *Journal of Nutrition*, **119,** 1062-1070.

Richardson, H. W. 1993. Cobalt compounds. In: *Encyclopaedia of Chemical Technology,* (Ed. by Kroschwitz, J. I. & Howe-Grant, M.), pp. 778. New York: John Wiley & Sons.

Ricklefs, R. E. 1977. Composition of eggs of several bird species. *The Auk*, **94,** 350-356.

Ricklefs, R. E. 1984. Components of variance in measurements of nestling European starlings (*Sturnus vulgaris*) in southeastern Pennsylvania. *Auk*, **101,** 319-333.

Ricklefs, R. E., Hahn, D. C. & Montevecchi, W. A. 1978. The relationship between egg size and chick size in the Laughing Gull and Japanese Quail. *The Auk*, **95,** 135-144.

Ridgway, L. P. & Karnofsky, D. A. 1952. The effects of metals on the chick embryo: toxicity and production of abnormalities in development. *Annals of the New York Academy of Sciences*, **55,** 203-215.

Risch, T. S. & Rohwer, F. C. 2000. Effects of parental quality and egg size on growth and survival of herring gull chicks. *Canadian Journal of Zoology*, **78,** 967-973.

Robbins, C. T. 1981. Estimation of the relative protein costs of reproduction in birds. *Condor*, **83,** 177-179.

Robinson, B. H., Bischofberger, S., Stoll, A., Schroer, D., Furrer, G., Roulier, S., Gruenwald, A., Attinger, W. & Schulin, R. 2008. Plant uptake of trace elements on a Swiss military shooting range: uptake pathways and land management implications. *Environmental Pollution*, **153,** 668-676.

Romanoff, A. L. & Romanoff, A. J. 1949. *The Avian Egg*. New York, USA: J. Wiley and Sons.

Rosenius, P. 1942. *Sveriges fåglar och fågelbon*. Lund, Sweden: Gleerups.

Rosenzweig, M. L. 1991. Habitat selection and populations: the search for mechanism. *American Naturalist*, **137,** S5-S28.

Rousseau, C., Barauda, F., Leleytera, L. & Gila, O. 2009. Cathodic protection by zinc sacrificial anodes: Impact on marine sediment metallic contamination *Journal of Hazardous Materials*, **167,** 953-958.

Rule, K. L., Comber, S. D. W., Ross, D., Thornton, A., Makropoulos, C. K. & Rautiu, R. 2006. Diffuse sources of heavy metals entering an urban wastewater catchment. *Chemosphere*, **63,** 64-72.

RWE npower. 2004a. Fawley Power Station - Visitor Leaflet. Rheinisch-Westfälisches Elektrizitätswerk npower.

RWE npower. 2004b. Fawley Power Station - Site Information. Rheinisch-Westfälisches Elektrizitätswerk npower.

Ryder, J., Orr, E. & Saedi, H. 1977. Egg quality in relation to nest location in Ring-billed Gulls. *Wilson Bulletin*, **89,** 473-475.

Sahin, N., Onderci, M. & Sahin, K. 2002. Effects of dietary chromium and zinc on egg production, egg quality, and some blood metabolites of laying hens reared under low ambient temperature *Biological Trace Element Research*, **85,** 47-58.

Saleem, Z. A. 1999. Summary of comments on: management and disposal of lead based paint debris. Washington DC: United States Environmental Protection Agency, .

Saliva, J. E. & Burger, J. 1989. Effect of experimental manipulation of vegetation density on nest-site selection in Sooty Terns. *The Condor*, **91,** 689-698.

Sanchez, J., Garcia, F. & Planas, J. 1987. Iron content and distribution in Japanese quail. *Quarterly Journal of Experimental Physiology* **72,** 21-29.

Sanger, D. M., Holland, A. F. & Scott, G. I. 1999. Tidal creek and salt marsh sediments in South Carolina coastal estuaries: I. Distribution of trace metals. *Archive of Environmental Contamination and Toxicology*, **37,** 445-457.

Sanz, J. J. 1998. Effects of geographic location and habitat on breeding parameters of Great Tits. *The Auk*, **115,** 1034-1051.

Scheuhammer, A. M. 1987. The chronic toxicity of aluminium, cadmium, mercury, and lead in birds: a review. *Environmental Pollution*, **46,** 263-295.

Schifferli, L. 1973. The effect of egg-weight on the subsequent growth of nestling great tits, *Parus major*. *Ibis*, **115,** 549-558.

Schnellman, G. A. & Scott, B. 1970. Lead-zinc mining areas of Great Britain. In: *Proceedings of the Ninth Commonwealth Mining and Metallurgy Congress 1969*, pp. 325-356.

Schreiber, E. A. & Burger, J. 2002. Seabirds in the marine environment. In: *Biology of Marine Birds* (Ed. by Schreiber, E. A. & Burger, J.), pp. 1-15. Boca Raton: CRC Press LLC.

Schroeder, H. A., Nason, A. P., Tipton, I. H. & Balassa, J. J. 1966. Essential trace metals in man: copper. *Journal of Chronic Diseases*, **19,** 1007-1034.

Schulz-Baldes, M., Rehm, E. & Farke, H. 1983. Field experiments on the fate of lead and chromium in an intertidal benthic mesocosm, the Bremerhaven Caisson. *Marine Biology*, **75,** 307-318.

SCOPAC. 2004a. Hurst Spit to Calshot Spit (Western Solent Mainland): Sediment sinks and stores - mudflats and saltmarshes. http://www.scopac.org.uk/scopac%20sediment%20db/wsol/wsol.htm: Standing Conference on Problems Associated with the Coastline.

SCOPAC. 2004b. Poole Harbour: Saltmarsh. http://www.scopac.org.uk/scopac%20sediment%20db/pharb/pharb.htm: Standing Conference on Problems Associated with the Coastline.

Scott, H. M. & Warren, D. C. 1941. The relation of total weight and the weight of the component parts of the egg to hatchery power. *Poultry Science*, **20,** 75-78.

Sea Grant. 2009. Greenwich Bay: Pollutants. www.seagrant.gso.uri.edu/G_Bay/HabitatEco/WaterQuality/pollutants.html: Rhode Island Sea Grant, University of Rhode Island Bay, School of Oceanography.

Segura, M., Madrid, Y. & Camara, C. 2003. Elimination of calcium and argon interferences in iron determination by ICP-MS using desferrioxamine chelating agent immobilized in sol-gel and cold plasma conditions. *Journal of analytical atomic spectrometry*, **18,** 1103-1108.

Sell, J. L. 1975. Cadmium and the laying hen: apparent absorption, tissue distribution and virtual absence of transfer into eggs. *Poultry Science*, **54,** 1674-1678.

Seyler, P. & Martin, J. M. 1989. Biogeochemical processes affecting arsenic species distribution in a permanently stratified lake. *Environmental Science and Technology*, **23,** 1258-1263.

Shannon, L. J. & Crawford, R. J. M. 1999. Management of the African penguins *Spheniscus demersus* - insights from modelling. *Marine Ornithology*, **27,** 119-128.

Sharma, V. K. & Millero, F. J. 1988. The oxidation of Cu(I) in Seawater. *Environmental Science and Technology*, **22,** 768-771.

Shields, W. M., Crook, W. M., Hebblethwaite, M. L. & Wiles-Ehmann, S. S. 1988. Ideal free coloniality in the swallows. In: *The Ecology of Social Behaviour* (Ed. by Slobodchikoff, C. N.), pp. 189-228. New York: Academic Press.

Siegel-Causey, D. & Kharitonov, S. P. 1990. The evolution of coloniality. In: *Current Ornithology* (Ed. by Johnson, R. F.), pp. 285-330. New York: Plenum Pres.

Sierra, P., Chakrabarti, S., Tounkara, R., Loranger, S., Kennedy, G. & Zayed, J. 1998. Bioaccumulation of manganese and its toxicity in feral pigeons (*Columba livia*) exposed to manganese oxide dust (Mn_3O_4). *Environmental Research*, **79,** 94-101.

Simkiss, K. 1980. Eggshell porosity and the water metabolism of the chick embryo. *Journal of Zoology*, **192,** 1-8.

Smith, I. C. & Carson, B. L. 1981. Trace Metals in the Environment. Volume 6 - Cobalt. Ann Arbor Science.

Smith, S. M. 1974. Nest-site selection in Black-capped Chickadees. *Condor*, **76,** 178-179.

Solent Forum. 2005a. Indicators for the health of the Solent. Winchester: Solent Forum.

Solent Forum. 2005b. Solent Forum Maps: http://www.solentforum.hants.org.uk/solentmaps/solent_maps.htm.

Solent Protection Society. 2006. West Solent Marine National Park Report. http://www.solentprotection.org/articles/46-west-solent-marine-national-park-report.

Sörme, L. & Lagerkvist, R. 2002. Sources of heavy metals in urban wastewater in Stockholm. *Science of the Total Environment*, **298,** 131-145.

Spahn, S. A. & Sherry, T. W. 1999. Cadmium and lead exposure associated with reduced growth rates, poorer fledging success of Little Blue Heron chicks (*Egretta caerulea*) in South Louisiana Wetlands. *Archive of Environmental Contamination and Toxicology*, **37,** 377-384.

Spallholz, J. E. & Hoffman, D. J. 2002. Selenium toxicity: cause and effects on aquatic birds. *Aquatic Toxicology*, **57,** 27-37.

Spear, L. B. 1993. Dynamics and effect of western gulls feeding in a colony of guillemots and Brandt's cormorants. *Journal of Animal Ecology*, **62,** 399-414.

Spencer, D. W. & Brewer, P. 1971. Vertical advection diffusion and redox potentials as controls of the distribution of manganese and other trace elements dissolved in waters of the Black Sea. *Journal of Geophysical Research* **36,** 5877-5892.

Spencer, K. L. & MacLeod, C. L. 2002. Distribution and partitioning of heavy metals in estuarine sediment cores and implications for the use of sediment quality standards. *Hydrology and Earth System Sciences*, **6,** 989-998.

Stanley, T. R., Spann, J. W., Smith, G. J. & Rosscoe, R. 1994. Main and interactive effects of arsenic and selenium on mallard reproduction and duckling growth and survival. *Archive of Environmental Contamination and Toxicology*, **26,** 444-451.

Stewart, F. M., Thompson, D. R., Furness, R. W. & Harrison, N. 1994. Seasonal variation in heavy metal levels in tissues of common guillemots *Uria aalge* from Northwest Scotland. *Archives of Environmental Contamination and Toxicology*, **27,** 168-175.

Stiff, M. J. 1971. The chemical states of copper in polluted fresh water and a scheme of analysis to differential them. *Water Research*, **5,** 585-599.

Stock, M., Herber, R. F. M. & Geron, H. M. A. 1989. Cadmium levels in oystercatcher *Haematopus ostralegus* from the German Wadden Sea. *Marine Ecology Progress Series*, **53,** 227-234.

Stokes, D. L. & Boersma, P. D. 2000. Nesting density and reproductive success in a colonial seabird, the Magellanic Penguin. *Ecology*, **81,** 2878-2891.

Stoneburner, D. L. & Harrison, C. S. 1981. Heavy metal residues in sooty tern tissues from the Gulf of Mexico and north central Pacific Ocean.

Stoneburner, D. L., Patty, P. C. & Robertson, W. B. 1980. Evidence of heavy metal accumulation in sooty terns. *Science of the Total Environment*, **14,** 147-152.

Styrsky, J. D., K.P., E. & Thompson, C. F. 1999. Fitness-related consequences of egg mass in nestling house wrens. *Proceedings of the Royal Society London: Biological Sciences*, **267,** 1253-1258.

Supplee, W. C. 1964. Observations on the effect of copper additions to purified turkey diets. *Poultry Science*, **43,** 1599-1600.

Svensson, S. 2002. Weight and size of eggs in the Black-Headed Gull *Larus ridibundus*. *Ornis Svecica*, **12,** 170-173.

Sydeman, W. J. & Emslie, S. D. 1992. Effects of parental age on hatching asynchrony, egg size and third-chick disadvantage in western gulls. *Auk*, **109,** 242-248.

Sydeman, W. J., Penniman, J. F., Penniman, T. M., Pyle, P. & Ainley, D. G. 1991. Breeding performance in the western gull: effects of parental age, timing of breeding and year on relation to food availability. *Journal of Animal Ecology*, **60,** 135-149.

Szefer, P., Szefer, K., Glasby, G. P., Pempkowiak, J. & Kaliszan, R. 1996. Heavy-metal pollution in surficial sediments from the southern Baltic Sea off Poland. *Journal of Environmental Science and Health, Part A*, **31,** 2723-2754.

Tanabe, S. 2002. Contamination and toxic effects of persistent endocrine disrupters in marine mammals and birds. *Marine Pollution Bulletin*, **45,** 69-77.

Tanabe, S., Subramanian, A., Hidaka, H. & Tatsukawa, R. 1986. Transfer rates and pattern of PCB isomers and congeners and pp-DDE from mother to egg in Adelie penguin (*Pygoscelis adeliae*). *Chemosphere*, **15,** 343-351.

Taulis, M. E. 2005. Metal contaminants in leachate from sanitary landfills. In: *Metal Contaminants in New Zealand* (Ed. by Moore, T. A., Black, A., Centeno, J. A., Harding, J. S. & Trumm, D. A.), pp. 173-190. Christchurch, New Zealand: Resolutionz Press.

Teesdale.co.uk. 2009. Middleton - Market Town. http://www.teesdale.co.uk/page.php?id=67.

Tenaza, R. 1971. Behaviour and nesting success relative to nest location in Adélie Penguins (*Pygoscelis adeliae*). *Condor*, **73,** 81-92.

Thompson, D. R. 1990. Metal levels in marine vertebrates. In: *Heavy Metals in the Marine Environment* (Ed. by Furness, R. W. & Rainbow, P. S.), pp. 143-182. Boca Raton, Florida: CRC Press.

Thornton, I. & John, S. W. 1980. Regional distribution of trace element problems in Great Britain. In: *Applied soil trace elements* (Ed. by Davies, B. E.), pp. 381-439. New York: John Wiley & Sons Ltd.

Thyen, S., Becker, P. H. & Behmann, H. 2000. Organochlorine and mercury contamination of little terns (*Sterna albifrons*) breeding at the western Baltic Sea, 1978-96. *Environmental Pollution*, **108,** 225-238.

Tillman, D. A. 1994. *Trace Metals in Combustion Systems*. New York: Academic Press.

Townend, I. 2008. A conceptual model of Southampton Water. http://www.estuary-guide.net/pdfs/southampton_water_case_study.pdf: Environment Agency, Defra and ABP-mer.

Townend, J. 2002. *Practical Statistics for Environmental and Biological Scientists*. London, U.K.: John Wiley & Sons Ltd.

Tuan, R. S., Ono, T., Akins, R. E. & Koide, M. 1991. Experimental studies on cultured, shell-less fowl embryos: calcium transport, skeletal development, and cardio-vascular functions. In: *Egg Incubation: Its Effect on Embryonic Development in Birds and Reptiles* (Ed. by Deeming, D. C. & Ferguson, M. W. J.), pp. 419-433. Cambridge: Cambridge University Press.

Tubbs, C. R. 1999. *The Ecology, Conservation and History of the Solent*. Chichester, Sussex: Packard Publishing.

Turner, B. L., Baxter, R. & Whitton, B. A. 2003. Nitrogen and phosphorus in soil solutions and drainage streams in Upper Teesdale, northern England: implications of organic compounds for biological nutrient limitation. *The Science of the Total Environment*, **314-316,** 153-170.

Turner, J. C., Solly, S. R. B., Mol-Krijnen, J. C. M. & Shanks, V. 1978. Organochlorine, fluorine, and heavy-metal levels in some birds from New Zealand estuaries. *New Zealand Journal of Science*, **21,** 99-??

UK Marine SAC. 2009. Toxic Substance Profile: Zinc. http://www.ukmarinesac.org.uk/activities/water-quality/wq8_6.htm

UK Ports. 2010. Ports and Harbours of the UK: Marchwood. http://www.ports.org.uk/port.asp?id=130.

UKPIA. 2008. ExxonMobil Refinery Fawley. http://www.ukpia.com/industry_information/refining_uk_refineries/refineries/exxonmobil_refinery_fawley.aspx. UK Petroleum Industry Association (UKPIA) Ltd.

Underwood, E. J. 1971. Manganese. In: *Trace Elements in Human and Animal Nutrition*, pp. 177-207. New York: Academic Press.

Underwood, E. J. & Suttle, N. F. 1999. *The Mineral Nutrition of Livestock*. New York: CABL.

USEPA. 1980a. Ambient water quality criteria for lead. In: *Report 440/5-80-057*. Virginia, USA: US Environmental Protection Agency.

USEPA. 1980b. Ambient water quality criteria for arsenic. In: *Report 440/5-80-021*, pp. 205. Virginia, USA: US Environmental Protection Agency.

USEPA. 1980c. Ambient water quality criteria for copper. In: *Report 440/5-80-036*. Virginia, USA: US Environmental Protection Agency.

USEPA. 1985. Ambient water quality criteria for arsenic - 1984. In: *Report 440/5-84-033*, pp. 66. Virginia, USA: US Environmental Protection Agency.

USEPA. 1993. Provisional guidance for quantitative risk assessment of polycyclic aromatic hydrocarbons. In: *EPA/600/R-93/089*. Cincinnati, Ohio: Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, US Protection Agency.

USEPA. 1996. Method 3052: Microwave assisted acid digestion of siliceous and organically based matrices. US Environmental Protection Agency.

USEPA. 2003. Technical summary of information available on the bioaccumulation of arsenic in aquatic organisms. Washington, DC: US Environmental Protection Agency: http://www.epa.gov/waterscience/criteria/arsenic/tech-sum-bioacc.pdf.

Van Bree, P. J. H. 1957. Variations in length and breadth of eggs from a colony of black-headed gulls (*Larus r. ridibundus* Linnaeus) on the island of Texel. *Beaufortia*, **5,** 245-255.

Van den Steen, E., Dauwe, T., Covaci, A., Jaspers, V. L. B., Pinxten, R. & Eens, M. 2006. Within- and among-clutch variation of organohalogenated contaminants in eggs of great tits (*Parus major*). *Environmental Pollution*, **144,** 355-359.

Van Vleet, J. F., Boon, G. D. & Ferrans, V. J. 1981. Induction of lesions of selenium-vitamin E deficiency in ducklings fed silver, copper, cobalt, tellurium, cadmium or zinc: protection by selenium or vitamin E supplements. *American Journal of Veterinary Research*, **42,** 1206-1217.

Vaughan, D. J. & Ixer, R. A. 1980. Studies of sulphide mineralogy of north Pennine ores and its contribution to genetic models. *Transactions of the Institute of Mining and Metallurgy (Section B: Applied Earth Sciences)*, **89,** B99-B110.

Veasey, J. S., Houston, D. C. & Metcalfe, N. B. 2000. Flight muscle atrophy and predation risk in birds. *Functional Ecology*, **14,** 115-121.

Veasey, J. S., Houston, D. C. & Metcalfe, N. B. 2001. A hidden cost of reproduction: the trade-off between clutch size and escape take-off speed in female zebra finches. *Journal of Animal Ecology*, **70,** 20-24.

Verheyen, E., Helsen, J. & Decuypere, E. 1990. Accumulation of zinc in egg yolk ovarian follicles and organs after forced resting by high dietary zinc. *British Poultry Science*, **31,** 147-154.

Verhulst, S. & Timbergen, J. M. 1991. Experimental evidence for a causal relationship between timing and success of reproduction in the great tit *Parus m. major*. *Journal of Animal Ecology*, **60,** 269-282.

Vermeer, K. & Castilla, J. C. 1991. High cadmium residues observed during a pilot study in shorebirds and their prey downstream from the El Salvador copper mine, Chile. *Bulletin of Environmental Contamination and Toxicology*, **46,** 242-248.

Vermeer, K., Morgan, K. H., Smith, G. E. J. & York, B. A. 1991. Effects of egging on the reproduction success of Glaucous-winged gulls. *Waterbirds*, **14,** 158-165.

Vermeer, K. & Thompson, J. A. J. 1992. Arsenic and copper residues in waterbirds and their food down inlet from the Island Copper Mil. *Bulletin of Environmental Contamination and Toxicology*, **48,** 733-738.

Vernon, J. D. R. 1972. Feeding habitats and food of the Black-headed and Common gulls. Part 2 - Food. *Bird Study*, **19,** 173-186.

Verrett, M. J. 1973. Investigation of the toxic and teratogenic effects of GRAS substances to the developing chick embryo: copper gluconate. Washington, DC: US Food and Drug Administration.

Verrett, M. J. 1974. Investigation of the toxic and teratogenic effects of GRAS substances to the developing chick embryo: copper gluconate, supplement. Washington, DC: US Food and Drug Administration.

Verrett, M. J. 1976. Investigations of the toxic and teratogenic effects of GRAS substances to the developing chick embryo: cupric chloride. Washington, DC: US Food and Drug Administration.

Visit Cumbria. 2008. Cow Green Reservoir, North Pennines. http://www.visitcumbria.com/pen/cowgreen.htm.

Visschedijka, H. & Rahn, H. 1981. Incubation of chicken eggs at altitude: theoretical consideration of optimal gas composition. *British Poultry Science*, **22,** 451-460.

Wagemann, R., Snow, N. B., Rosenberg, D. M. & Lutz, A. 1978. Arsenic in sediments, water and biota from lakes in the vicinity of Yellowknife, Northwest Territories Canada. *Archive of Environmental Contamination and Toxicology*, **7,** 169-191.

Wallner-Pendleton, E., Froman, D. P. & Hedstrom, O. 1986. Identification of ferrous sulfate toxicity in a commercial broiler flock. *Avian Diseases*, **30,** 430-432.

Walsh, P. M. 1988. Geographical variation in mercury levels of North Atlantic gannets *Sula bassana*. In: *3rd Int. Conf. Seabird Group*. Cambridge, UK.

Walsh, P. M. 1990. The use of seabirds as monitors of heavy metals in the marine environment. In: *Heavy Metals in The Marine Environment* (Ed. by Furness, R. W. R., P.S.), pp. 183-204. New York: CRC Press.

Walsh, P. M., Halley, D. J., Harris, M. P., del Nevo, D., Sim, I. M. W. & Tasker, M. L. 1995. *Seabird Monitoring Handbook for Britain and Ireland*. Peterborough: Joint Nature Conservation Commitee.

Wanless, S., Harris, M. P., Calladine, J. & Rothery, P. 1996. Modelling responses of herring gull and lesser black-backed gull populations to reduction of reproductive output: implications for control measures. *Journal of Applied Ecology*, **33,** 1420-1432.

Ward, P. & Zahavi, A. 1973. The importance of certain assemblages of birds as "information centres" for food finding. *Ibis*, **115,** 517-534.

watertreatment.com. 2006. Swanage Membrane Bioreactor (MBR) Wastewater Treatment Plant.

Weidmann, U. 1956. Observations and experiments on egg-laying in the Black-headed gull (*Larus ridibundus* L.). *British Journal of Animal Behaviour*, **4,** 150-161.

Weyers, B., Gluck, E. & Stoeppler, M. 1988. Investigation of the significance of heavy metal contents of blackbird feathers. *Science of the Total Environment*, **77,** 61-67.

White, D. H. & Dieter, M. P. 1978. Effects of dietary vanadium in mallard ducks. *Journal of Toxicology and Environmental Health*, **4,** 43-50.

White, D. H. & Finley, M. T. 1978. Uptake and retention of dietary cadmium on kidneys and testes of mallard ducks. *Environmental Research*, **17,** 53-59.

White, D. H., King, K. A. & Prouty, R. M. 1980. Significance of organochlorine and heavy metal residues in wintering shorebirds at Corpus Christi, Texas, 1976–77 *Pesticides Monitoring Journal*, **14,** 58-63.

WHO. 1981. Environmental Health Criteria 17: manganese. In: *Environmental Health Criteria*. Geneva: International Programme on Chemical Safety (IPCS), World Health Organisation.

WHO. 1986. Environmental Health Criteria 58: selenium. In: *Environmental Health Criteria*. Geneva: International Programme on Chemical Safety (IPCS), World Health Organisation.

WHO. 1989. Environmental Health Criteria 85: lead - environmental aspects. In: *Environmental Health Criteria*. Geneva: International Programme on Chemical Safety (IPCS), World Health Organisation.

WHO. 1991. Environmental Health Criteria 108: nickel. In: *Environmental Health Criteria*. Geneva: International Programme on Chemical Safety (IPCS), World Health Organisation.

WHO. 1998. Environmental Health Criteria 200: copper. In: *Environmental Health Criteria*. Geneva: International Programme on Chemical Safety (IPCS), World Health Organisation.

WHO. 2001a. Concise International Chemical Assessment Document 29: vanadium pentoxide and other inorganic vanadium compounds. In: *Concise International Chemical Assessment Document*. Geneva: International Programme on Chemical Safety (IPCS), World Health Organisation.

WHO. 2001b. Environmental Health Criteria 221: zinc. In: *Environmental Health Criteria*. Geneva: International Programme on Chemical Safety (IPCS), World Health Organisation.

WHO. 2004a. Concise International Chemical Assessment Document 63: manganese and its compounds - environmental aspects. In: *Concise International Chemical Assessment Document*. Geneva: International Programme on Chemical Safety (IPCS), World Health Organisation.

WHO. 2004b. Food Additive Series 18: iron. In: *Food Additive Series*. Geneva: International Programme on Chemical Safety (IPCS), World Health Organisation.

WHO. 2006. Concise International Chemical Assessment Document 69: cobalt and inorganic cobalt compounds. In: *Concise International Chemical Assessment Document*. Geneva: International Programme on Chemical Safety (IPCS), World Health Organisation.

Wiebe, K. L. & Bortolotti, G. R. 1995. Food-dependent benefits of asynchronous hatching in American kestrels. *Behavioural Ecology and Sociobiology*, **36,** 49-57.

Wiemeyer, S. N., Lamont, T. G., Bunck, C. M., Sindelar, C. R., Cramlich, F. J., Fraser, J. D. & Byrd, M. A. 1984. Organochlorine pesticide, polychlorobiphenyl, and mercury residues in bald eagle eggs 1969-1979 and their relationships to shell thinning and reproduction. *Archives of Environmental Contamination and Toxicology*, **13,** 529-549.

Wilgus, H. S., Norris, L. C. & Heuser, G. F. 1936. The role of certain inorganic elements in the cause and prevention of perosis. *Science*, **84,** 252-253.

Williams, E. C. R. *in preparation*. The impact of changing sediment budgets on the intertidal zone around an active port: a case study of Southampton Water, UK. In: *Centre for Environmental Sciences, School of Engineering and the Environment*: University of Southampton.

Williams, E. C. R., Bray, S., Lloyd-Jones, D., Steyl, I., Hudson, M. & Nicholls, R. J. 2009. Scoping study site analysis for potential beneficial dredge spoil use for restoration and recharge of intertidal soft sediment resources within the Solent. pp. 132. Southampton: University of Southampton.

Williams, L., Schoof, R. A., Yager, J. W. & Goodrich-Mahoney, J. W. 2006. Arsenic bioaccumulation in freshwater fishes. *Human and Ecological Risk Assessment*, **12,** 904-923.

Williams, M., Hothem, R. L. & Ohlendorf, H. M. 1989a. Recruitment failure in American avocets and black-necked stilts nesting at Kesterson Reservoir, California, 1984-1985. *Condor*, **91,** 797-802.

Williams, S. N., Miles, R. D., Ouart, M. D. & Campbell, D. R. 1989b. Short-term high level zinc feeding and tissue zinc concentration in mature laying hens. *Poultry Science*, **68,** 539-545.

Williams, T. D. 1994. Intraspecific variations in egg size and egg composition in birds: effects on offspring fitness. *Biological Reviews*, **68,** 35-59.

Wood, P. J. 2007. PhD: Human impacts on coastal bird populations in the Solent. In: *School of Civil Engineering and the Environment, University of Southampton*. Southampton.

Wood, P. J., Hudson, M. D. & Doncaster, C. P. 2009. Impact of egg harvesting on breeding success of black-headed gulls, *Larus ridibundus Acta Oecologica*, **35,** 83-93.

Woolson, E. A. 1975. *Arsenical Pesticides*. American Chemical Society Symposium, Series 7.

Yahner, R. H. 1983. Site-related nesting success of Mourning Doves and American Robins in Shelterbelts. *Wilson Bulletin*, **95,** 573-580.

Yamauchi, H., Kaise, T., Takahashi, K. & Yamamura, Y. 1990. Toxicity and metabolism of trimethylarsine in mice and hamster. *Fundamental and Applied Toxicology*, **14,** 399-407.

Yannakopoulos, A. L., Tserveni-Gousi, A. S. & Christaki, E. 1998. Effect of natural zeolite on yolk:albumen ratio in hen eggs. *British Poultry Science*, **39,** 506-510.

Yorio, P., Bertellotti, M. & Quintana, F. 1995. Preference for covered nest sites and breeding success in Kelp Gulls *Larus dominicanus*. *Marine Ornithology*, **23,** 121-128.

Young, B., Styles, M. B. & Berridge, N. G. 1985. Niccolite magnetite mineralization from Upper Teesdale, North Pennines. *Mineralogical Magazine*, **49,** 555-559.

Ytreberg, N.-J. 1956. Contribution to the breeding biology of the Black-headed Gull (*Larus ridibundus* L.) in Norway. *Nytt Magasin for Zoologi*, **4,** 5-106.

Zador, S. G., Piatt, J. F. & Punt, A. E. 2005. Balancing predation and egg harvest in a colonial seabird: a simulation model. *Ecological Modelling*, **195,** 318-326.

Zhanga, H. H., Lia, F. B., Wua, Z. F., Lia, D. Q., Xub, D. R. & Yuana, H. X. 2008. Baseline concentrations and spatial distribution of trace metals in surface soils of Guangdong Province, China. *Journal of Environmental Quality*, **37,** 1752-1760.

Zhu, J. & Zheng, B. 2001. Distribution of selenium in a mini-landscape of Yutangba, Enshi, Hubei Province, China *Applied Geochemistry*, **16,** 1333-1344.

Zoumis, T., Schmidt, A., Grigorova, L. & Calmano, W. 2001. Contaminants in sediments: remobilisation and demobilisation. *Science of the Total Environment*, **266,** 195-202.

APPENDIX A: PEARSON'S CORRELATION ANALYSIS FOR NEST DENSITY STUDY

Table A.1 Pearson's correlation analysis for egg size with nest density: Lymington site (n = 20)

Egg	Measurement	Radius (m)	r	p	Relationship
Egg 1	Length	2	0.290	0.070	none
	Breadth	2	0.003	0.986	none
	Volume	2	0.129	0.427	none
	Length	5	0.273	0.088	none
	Breadth	5	-0.013	0.937	none
	Volume	5	0.111	0.494	none
Egg 2	Length	2	0.257	0.110	none
	Breadth	2	-0.056	0.730	none
	Volume	2	0.084	0.605	none
	Length	5	0.270	0.092	none
	Breadth	5	-0.041	0.799	none
	Volume	5	0.103	0.526	none
Egg 3	Length	2	0.020	0.904	none
	Breadth	2	-0.020	0.901	none
	Volume	2	0.000	0.998	none
	Length	5	0.118	0.467	none
	Breadth	5	-0.032	0.843	none
	Volume	5	0.027	0.870	none

Table A.2 Pearson's correlation analysis for egg size with nest density: Poole site (n = 20)

Egg	Measurement	Radius (m)	r	p	Relationship
Egg 1	Length	2	0.068	0.678	none
	Breadth	2	-0.142	0.383	none
	Volume	2	0.028	0.866	none
	Length	5	0.292	0.068	none
	Breadth	5	0.023	0.889	none
	Volume	5	0.091	0.575	none
Egg 2	Length	2	-0.160	0.324	none
	Breadth	2	-0.103	0.527	none
	Volume	2	-0.122	0.455	none
	Length	5	0.029	0.861	none
	Breadth	5	-0.001	0.997	none
	Volume	5	0.020	0.902	none
Egg 3	Length	2	0.004	0.981	none
	Breadth	2	0.005	0.978	none
	Volume	2	-0.128	0.432	none
	Length	5	0.037	0.82	none
	Breadth	5	-0.017	0.917	none
	Volume	5	0.040	0.809	none

APPENDIX B: INDUSTRY AND WASTE AROUND THE LYMINGTON, POOLE AND RABY SITES

Water Industry
Waste Processes
Fuel and Power Production
Chemical Industry
Metal Production and Processing
Radioactive Substance Sites
Mineral Industry
Other Industry

Table B.1 Industry and waste sites around the Lymington colony

Map Ref no.	Company/site name	Industry type
1	Christchurch STW	
2	Pennington STW	
3, 4, 5	Efford Landfill	
6	Contract Heat and Power Ltd.	
7	SP Systems	
8	Fairlee STW	
9	Lyn Bottom Landfill	
10	Cowes Power (Gas Turbine) Station	
11	GKN Westland Aerospace (Holdings) Ltd.	
12	Poligrat UK Ltd.	
13	Fawley Power Station	
14	NPower Cogen Ltd., Esso Refinery	
15	EXXON Mobil Chemical Ltd.	
16	Cognis UK Ltd.	
17	BP CHP (UK) Ltd.	
18	Ondeo Nalco Ltd.	
19	Polimeri Europe UK Ltd.	
20	BP Oil (UK) Ltd., Hamble Oil Terminal	
21	Aerostructures Hamble Ltd.	
22	Woolston STW	
23	Marchwood Industrial Estate	
24	University of Southampton National Oceanography Centre	
25	Slowhill Copse STW	
26	Holmsley Pit Landfill	
27	Selex Sensors and Airbourne Systems Infrared Ltd.	
28	Millbrook STW	
29	A and P Southampton Ltd., Coating, Printing, Textiles	
30	Bacardi Martini Ltd., Animal, Vegetable and Food	
31	Royal Bournemouth and Christchurch NHS Hospitals	
32	Koppers UK Ltd.	
33	Rank Hovis Ltd., Solent Flour Mills	
34	Morgan Advanced Ceramics Ltd.	
35	SPI Lasers	
36	Ryvan Chemical Co. Ltd.	
37	Portswood STW	
38	University of Southampton, Boldrewood Campus	
39	Southampton University Hospitals NHS Trust	
40	BUPA Hospitals	
41	Ringwood STW	
42	Bournemouth (Holdenhurst) STW	
44	HMS Daedalus	

Table B.2 Industry and waste sites around the Poole colony

Map Ref no.	Company/site name	Industry type
1	AEA Technology Plc.	
2	Viridor Waste Management Ltd.	
3	AMEC Nuclear UK Ltd.	
4	Centre for Ecology and Hydrology	
5	Ministry of Defence	
6	UKAEA and NUVIA Ltd.	
7, 8	BP Exploration Operating Co. Ltd.	
9	Swanage STW	
10, 11	BP Exploration Operating Co. Ltd., Wytch Farm	
12, 13	Tatchells Landfill	
14	Merck Ltd.	
15	Poole Technical Plating Systems Ltd.	
16	Bowmill Metal Treatments Ltd.	
17	Poole Hospital NHS Trust	
18	SITA Products and Service Ltd.	
19	SITA UK Ltd.	
20	Corfe Mullen Landfill	
21	OSS Group Ltd.	
22	Solvwaste Environmental Ltd.	
23	W H White Plc.	
24	Siemens Power Generation Radiation Monitoring	
25	Poole Real Estate Ltd.	
26	Poole STW	
27	Iracroft Ltd.	
28, 29	Sigma-Aldrich Co. Ltd.	
30	Magellan Aerospace Bournemouth Ltd.	
31	University of Bournemouth, Talbot Campus	
32	Royal Bournemouth and Christchurch Hospital	
33	Christchurch STW	
34	Bournemouth (Holdenhurst) STW	
35	Bournemouth (Kinson) STW	
36	Flight Refuelling Ltd.	
37	Wimbourne STW	
38	Cleansing Service Group Ltd.	
39	Goldenfuels Ltd.	
40	Viridor Waste (Exeter) Ltd.	
41	Portsmouth Aviation Ltd.	
42	Palmersford STW	
43	Ringwood STW	
44	Chambers Runfold Plc.	
45	Onyx Landfiill Ltd.	
46	Heritage Farm Landfill	
47	Veolia ES Landfill Ltd.	
48	J & G Environmental Ltd.	
49	Faccenda Group Ltd., Brook Mill	

Table B.3 Industry and waste sites around the Raby colony

Map Ref no.	Company/site name	Industry type
1	British Gypsum Ltd.	
2	Appleby STW	
3	Blue Circle Cement	
4	Cotherstone Moor	

APPENDIX C: RECOVERY DATA FOR METALS IN EGG COMPONENTS

Note: recovery data highlighted in grey was not included in the calculation of average recovery

Table C.1 Recovery data for multi-element-spiked eggs: arsenic

Sample		As spiked (µg)	Spiked [As] in sample after treatment (µg/g)	[As] blank-corrected (µg/g)	Recovery (%)	Average recovery (%)
Yolk - spiked	1	10	2.762	2.557	92.5	88.2
	2	50	12.46	9.741	78.2	
	3	100	29.45	26.04	88.4	
	4	250	71.45	65.36	91.5	
	5	500	125.3	113.3	90.4	
Albumen - spiked	1	10	4.562	4.327	94.8	85.1
	2	50	21.87	18.97	86.8	
	3	100	41.31	38.97	94.3	
	4	250	96.04	83.65	87.1	
	5	500	242.6	151.7	62.5	
Shell - spiked	1	10	2.793	2.132	76.3	73.9
	2	50	13.21	10.98	83.1	
	3	100	39.62	26.49	66.9	
	4	250	64.32	45.64	71.0	
	5	500	128.8	93.24	72.4	

Table C.2 Recovery data for multi-element-spiked eggs: cadmium

Sample		Cd spiked (µg)	Spiked [Cd] in sample after treatment (µg/g)	[Cd] blank-corrected (µg/g)	Recovery (%)	Average recovery (%)
Yolk - spiked	1	2.5	0.691	0.661	95.7	96.2
	2	12.5	3.114	2.584	83.0	
	3	25	7.362	7.084	96.2	
	4	62.5	17.86	19.24	107.7	
	5	125	31.33	30.76	98.2	
Albumen - spiked	1	2.5	1.141	1.062	93.1	87.5
	2	12.5	5.468	4.936	90.3	
	3	25	10.33	10.02	97.1	
	4	62.5	24.01	22.55	93.9	
	5	125	60.65	38.42	63.3	
Shell - spiked	1	2.5	0.698	0.471	67.5	74.1
	2	12.5	3.303	2.634	79.7	
	3	25	9.905	6.792	68.6	
	4	62.5	16.08	12.63	78.6	
	5	125	32.20	24.54	76.2	

Table C.3 Recovery data for multi-element-spiked eggs: cobalt

Sample		Co spiked (µg)	Spiked [Co] in sample after treatment (µg/g)	[Co] blank-corrected (µg/g)	Recovery (%)	Average recovery (%)
Yolk - spiked	1	10	2.762	2.860	103.5	102.7
	2	50	12.46	10.84	87.0	
	3	100	29.45	29.60	100.5	
	4	250	71.45	83.35	116.7	
	5	500	125.3	132.6	105.8	
Albumen - spiked	1	10	4.562	4.724	103.6	94.6
	2	50	21.87	20.77	95.0	
	3	100	41.31	42.76	103.5	
	4	250	96.04	96.52	100.5	
	5	500	242.6	171.3	70.6	
Shell - spiked	1	10	2.793	2.122	76.0	86.1
	2	50	13.21	13.12	99.3	
	3	100	39.62	32.24	81.4	
	4	250	64.32	58.91	91.6	
	5	500	128.8	105.6	82.0	

Table C.4 Recovery data for multi-element-spiked eggs: copper

Sample		Cu spiked (µg)	Spiked [Cu] in sample after treatment (µg/g)	[Cu] blank-corrected (µg/g)	Recovery (%)	Average recovery (%)
Yolk - spiked	1	10	2.762	3.679	133.2	108.3
	2	50	12.46	11.07	88.9	
	3	100	29.45	31.09	105.6	
	4	250	71.45	78.80	110.3	
	5	500	125.3	129.6	103.4	
Albumen - spiked	1	10	4.562	3.974	87.1	85.9
	2	50	21.87	18.70	85.5	
	3	100	41.31	41.51	100.5	
	4	250	96.04	86.69	90.3	
	5	500	242.6	160.1	66.0	
Shell - spiked	1	10	2.793	1.971	70.5	79.9
	2	50	13.21	12.37	93.6	
	3	100	39.62	29.55	74.6	
	4	250	64.32	51.46	80.0	
	5	500	128.8	103.9	80.7	

Table C.5 Recovery data for multi-element-spiked eggs: iron

Sample		Fe spiked (µg)	Spiked [Fe] in sample after treatment (µg/g)	[Fe] blank-corrected (µg/g)	Recovery (%)	Average recovery (%)
Yolk - spiked	1	10	2.762	19.39	701.9	138.4
	2	50	12.46	13.03	104.6	
	3	100	29.45	46.72	158.7	
	4	250	71.45	111.9	156.6	
	5	500	125.3	167.3	133.5	
Albumen - spiked	1	10	4.562	ND	-	73.8
	2	50	21.87	8.273	37.8	
	3	100	41.31	31.82	77.0	
	4	250	96.04	75.97	79.1	
	5	500	242.6	158.7	65.4	
Shell - spiked	1	10	2.793	5.715	204.6	82.5
	2	50	13.21	17.16	129.8	
	3	100	39.62	27.12	68.4	
	4	250	64.32	38.59	60.0	
	5	500	128.8	92.60	71.9	

Table C.6 Recovery data for multi-element-spiked eggs: lead

Sample		Pb spiked (µg)	Spiked [Pb] in sample after treatment (µg/g)	[Pb] blank-corrected (µg/g)	Recovery (%)	Average recovery (%)
Yolk - spiked	1	10	2.762	2.980	107.9	106.9
	2	50	12.46	12.06	96.8	
	3	100	29.45	31.39	106.6	
	4	250	71.45	84.69	118.5	
	5	500	125.3	131.1	104.6	
Albumen - spiked	1	10	4.562	4.231	92.7	91.5
	2	50	21.87	20.26	92.6	
	3	100	41.31	42.93	103.9	
	4	250	96.04	97.99	102.0	
	5	500	242.6	161.1	66.4	
Shell - spiked	1	10	2.793	13.53	484.4	85.1
	2	50	13.21	12.32	93.3	
	3	100	39.62	30.61	77.3	
	4	250	64.32	52.42	81.5	
	5	500	128.8	113.6	88.2	

Table C.7 Recovery data for multi-element-spiked eggs: manganese

Sample		Mn spiked (µg)	Spiked [Mn] in sample after treatment (µg/g)	[Mn] blank-corrected (µg/g)	Recovery (%)	Average recovery (%)
Yolk - spiked	1	10	2.762	3.154	114.2	106.4
	2	50	12.46	10.98	88.2	
	3	100	29.45	31.03	105.4	
	4	250	71.45	83.30	116.6	
	5	500	125.3	135.1	107.8	
Albumen - spiked	1	10	4.562	4.541	99.5	94.0
	2	50	21.87	21.07	96.3	
	3	100	41.31	42.80	103.6	
	4	250	96.04	97.41	101.4	
	5	500	242.6	167.2	68.9	
Shell - spiked	1	10	2.793	2.042	73.1	82.9
	2	50	13.21	11.82	89.4	
	3	100	39.62	31.00	78.2	
	4	250	64.32	58.45	90.9	
	5	500	128.8	106.5	82.7	

Table C.8 Recovery data for multi-element-spiked eggs: nickel

Sample		Ni spiked (µg)	Spiked [Ni] in sample after treatment (µg/g)	[Ni] blank-corrected (µg/g)	Recovery (%)	Average recovery (%)
Yolk - spiked	1	10	2.762	3.337	120.8	105.7
	2	50	12.46	10.99	88.2	
	3	100	29.45	29.70	100.9	
	4	250	71.45	82.63	115.6	
	5	500	125.3	128.8	102.8	
Albumen - spiked	1	10	4.562	4.173	91.5	90.7
	2	50	21.87	20.50	93.7	
	3	100	41.31	42.18	102.1	
	4	250	96.04	94.46	98.4	
	5	500	242.6	164.8	67.9	
Shell - spiked	1	10	2.793	1.952	69.9	87.6
	2	50	13.21	14.48	109.6	
	3	100	39.62	33.26	84.0	
	4	250	64.32	60.18	93.6	
	5	500	128.8	104.3	81.0	

Table C.9 Recovery data for multi-element-spiked eggs: selenium

Sample		Se spiked (µg)	Spiked [Se] in sample after treatment (µg/g)	[Se] blank-corrected (µg/g)	Recovery (%)	Average recovery (%)
Yolk - spiked	1	2.5	0.691	0.771	111.7	93.1
	2	12.5	3.114	2.422	77.8	
	3	25	7.362	6.278	85.3	
	4	62.5	17.86	16.27	91.1	
	5	125	31.33	31.15	99.4	
Albumen - spiked	1	2.5	1.141	1.068	93.7	78.6
	2	12.5	5.468	4.379	80.1	
	3	25	10.33	8.562	82.9	
	4	62.5	24.01	18.43	76.8	
	5	125	60.65	36.05	59.4	
Shell - spiked	1	2.5	0.698	0.537	76.9	73.6
	2	12.5	3.303	2.277	68.9	
	3	25	9.905	6.750	68.2	
	4	62.5	16.08	12.57	78.2	
	5	125	32.20	24.45	75.9	

Table C.10 Recovery data for multi-element-spiked eggs: vanadium

Sample		V spiked (µg)	Spiked [V] in sample after treatment (µg/g)	[V] blank-corrected (µg/g)	Recovery (%)	Average recovery (%)
Yolk - spiked	1	25	6.906	8.475	122.7	103.9
	2	125	31.14	28.37	91.1	
	3	250	73.62	76.73	104.2	
	4	625	178.6	195.4	109.4	
	5	1250	313.3	347.6	111.0	
Albumen - spiked	1	25	11.41	11.41	100.1	92.2
	2	125	54.68	52.23	95.5	
	3	250	103.3	106.0	102.6	
	4	625	240.1	228.4	95.1	
	5	1250	606.5	409.3	67.5	
Shell - spiked	1	25	6.983	10.95	156.9	83.8
	2	125	33.03	31.71	96.0	
	3	250	99.05	75.82	76.6	
	4	625	160.8	129.3	80.4	
	5	1250	322.0	264.2	82.1	

Table C.11 Recovery data for multi-element-spiked eggs: zinc

Sample		Zn spiked (µg)	Spiked [Zn] in sample after treatment (µg/g)	[Zn] blank-corrected (µg/g)	Recovery (%)	Average recovery (%)
Yolk - spiked	1	10	2.762	13.44	486.5	100.6
	2	50	12.46	6.268	50.3	
	3	100	29.45	37.24	126.5	
	4	250	71.45	92.85	130.0	
	5	500	125.3	120.0	95.7	
Albumen - spiked	1	10	4.562	2.041	44.7	83.2
	2	50	21.87	27.97	127.9	
	3	100	41.31	37.63	91.1	
	4	250	96.04	82.59	86.0	
	5	500	242.6	176.0	72.5	
Shell - spiked	1	10	2.793	1.651	59.1	68.4
	2	50	13.21	10.58	80.0	
	3	100	39.62	23.74	59.9	
	4	250	64.32	46.96	73.0	
	5	500	128.8	90.16	70.0	

APPENDIX D: METAL CONCENTRATIONS IN WASHED VS UNWASHED FEATHERS

Table D.1 Arsenic concentrations in washed and unwashed feathers

Treatment		[As] µg/g dry wt	Mean [As] µg/g dry wt
Unwashed	1	1.05	0.31
	2	0.00	
	3	0.28	
	4	0.00	
	5	0.00	
	6	0.55	
Washed	1	0.17	0.08
	2	0.25	
	3	0.09	
	4	0.00	
	5	0.00	
	6	0.00	

Table D.2 Cadmium concentrations in washed and unwashed feathers

Treatment		[Cd] µg/g dry wt	Mean [Cd] µg/g dry wt
Unwashed	1	0.32	0.09
	2	0.10	
	3	0.10	
	4	0.00	
	5	0.00	
	6	0.00	
Washed	1	0.16	0.07
	2	0.11	
	3	0.13	
	4	0.00	
	5	0.00	
	6	0.00	

Table D.3 Cobalt concentrations in washed and unwashed feathers

Treatment		[Co] µg/g dry wt	Mean [Co] µg/g dry wt
Unwashed	1	0.97	0.22
	2	0.06	
	3	0.19	
	4	0.00	
	5	0.00	
	6	0.12	
Washed	1	0.27	0.07
	2	0.17	
	3	0.00	
	4	0.00	
	5	0.00	
	6	0.00	

Table D.4 Copper concentrations in washed and unwashed feathers

Treatment		[Cu] µg/g dry wt	Mean [Cu] µg/g dry wt
Unwashed	1	14.0	14.6
	2	9.31	
	3	10.9	
	4	22.12	
	5	13.53	
	6	17.64	
Washed	1	15.4	14.0
	2	13.3	
	3	65.2*	
	4	14.91	
	5	13.01	
	6	13.00	

Table D.5 Iron concentrations in washed and unwashed feathers

Treatment		[Fe] µg/g dry wt	Mean [Fe] µg/g dry wt
Unwashed	1	112	355
	2	279	
	3	665	
	4	330	
	5	164	
	6	578	
Washed	1	14.7	78.6
	2	55.3	
	3	0.00	
	4	158	
	5	88.8	
	6	155	

Table D.6 Lead concentrations in washed and unwashed feathers

Treatment		[Pb] µg/g dry wt	Mean [Pb] µg/g dry wt
Unwashed	1	5.48	7.58
	2	5.89	
	3	6.30	
	4	12.59	
	5	4.23	
	6	11.00	
Washed	1	3.38	4.27
	2	3.68	
	3	1.56	
	4	9.96	
	5	2.78	
	6	4.29	

Table D.7 Manganese concentrations in washed and unwashed feathers

Treatment		[Mn] µg/g dry wt	Mean [Mn] µg/g dry wt
Unwashed	1	22.3	37.4
	2	29.8	
	3	65.4	
	4	26.9	
	5	12.8	
	6	67.0	
Washed	1	5.67	9.49
	2	9.37	
	3	1.32	
	4	17.23	
	5	4.95	
	6	18.41	

Table D.8 Nickel concentrations in washed and unwashed feathers

Treatment		[Ni] µg/g dry wt	Mean [Ni] µg/g dry wt
Unwashed	1	1.51	0.60
	2	0.00	
	3	0.57	
	4	0.76	
	5	0.19	
	6	0.55	
Washed	1	0.50	0.18
	2	0.27	
	3	0.28	
	4	0.00	
	5	0.00	
	6	0.00	

Table D.9 Selenium concentrations in washed and unwashed feathers

Treatment		[Se] µg/g dry wt	Mean [Se] µg/g dry wt
Unwashed	1	0.58	0.37
	2	0.83	
	3	0.57	
	4	0.00	
	5	0.00	
	6	0.26	
Washed	1	0.00	0.25
	2	0.78	
	3	0.70	
	4	0.00	
	5	0.00	
	6	0.00	

Table D.10 Vanadium concentrations in washed and unwashed feathers

Treatment		[V] µg/g dry wt	Mean [V] µg/g dry wt
Unwashed	1	25.8	42.8
	2	38.1	
	3	41.1	
	4	53.0	
	5	37.3	
	6	61.5	
Washed	1	29.5	24.1
	2	23.7	
	3	26.2	
	4	20.4	
	5	29.0	
	6	15.5	

Table D.11 Zinc concentrations in washed and unwashed feathers

Treatment		[Zn] µg/g dry wt	Mean [Zn] µg/g dry wt
Unwashed	1	67.3	86.9
	2	74.4	
	3	78.8	
	4	111	
	5	83.9	
	6	106	
Washed	1	51.3	83.5
	2	79.4	
	3	83.5	
	4	105	
	5	62.8	
	6	119	

APPENDIX E: RECOVERY DATA FOR METALS IN FEATHERS

Table E.1 Recovery data for multi-element-spiked feathers: arsenic

Sample	As spiked (µg)	Spiked [As] in sample after treatment (µg/g)	[As] blank-corrected (µg/g)	Recovery (%)	Average recovery (%)
1	10	1333.3	885.3	66.4	
2	50	2193.0	1425.2	65.0	
3	100	6250.0	3411.4	54.6	71.7
4	250	8992.8	8176.6	90.9	
5	500	9881.4	8052.8	81.5	

Table E.2 Recovery data for multi-element-spiked feathers: cadmium

Sample	Cd spiked (µg)	Spiked [Cd] in sample after treatment (µg/g)	[Cd] blank-corrected (µg/g)	Recovery (%)	Average recovery (%)
1	2.5	333.3	238.508	71.6	
2	12.5	548.2	378.519	69.0	
3	25	1562.5	919.690	58.9	77.4
4	62.5	2248.2	2232.535	99.3	
5	125	2470.4	2185.275	88.5	

Table E.3 Recovery data for multi-element-spiked feathers: cobalt

Sample	Co spiked (µg)	Spiked [Co] in sample after treatment (µg/g)	[Co] blank-corrected (µg/g)	Recovery (%)	Average recovery (%)
1	10	1333.3	968.3	72.6	
2	50	2193.0	1581.7	72.1	
3	100	6250.0	3445.2	55.1	74.9
4	250	8992.8	8298.6	92.3	
5	500	9881.4	8128.4	82.3	

Table E.4 Recovery data for multi-element-spiked feathers: copper

Sample	Cu spiked (µg)	Spiked [Cu] in sample after treatment (µg/g)	[Cu] blank-corrected (µg/g)	Recovery (%)	Average recovery (%)
1	10	1333.3	948.9	71.2	
2	50	2193.0	1535.6	70.0	
3	100	6250.0	3739.2	59.8	77.8
4	250	8992.8	8797.6	97.8	
5	500	9881.4	8890.4	90.0	

Table E.5 Recovery data for multi-element-spiked feathers: iron

Sample	Fe spiked (µg)	Spiked [Fe] in sample after treatment (µg/g)	[Fe] blank-corrected (µg/g)	Recovery (%)	Average recovery (%)
1	10	1333.3	1126.3	84.5	
2	50	2193.0	1605.0	73.2	
3	100	6250.0	3636.5	58.2	80.4
4	250	8992.8	8906.3	99.0	
5	500	9881.4	8607.1	87.1	

Table E.6 Recovery data for multi-element-spiked feathers: lead

Sample	Pb spiked (µg)	Spiked [Pb] in sample after treatment (µg/g)	[Pb] blank-corrected (µg/g)	Recovery (%)	Average recovery (%)
1	10	1333.3	1083.6	81.3	
2	50	2193.0	1656.6	75.5	
3	100	6250.0	4049.6	64.8	**84.1**
4	250	8992.8	9892.3	110.0	
5	500	9881.4	9796.5	99.1	

Table E.7 Recovery data for multi-element-spiked feathers: manganese

Sample	Mn spiked (µg)	Spiked [Mn] in sample after treatment (µg/g)	[Mn] blank-corrected (µg/g)	Recovery (%)	Average recovery (%)
1	10	1333.3	1057.9	79.3	
2	50	2193.0	1560.6	71.2	
3	100	6250.0	3833.3	61.3	**81.4**
4	250	8992.8	9281.8	103.2	
5	500	9881.4	9087.4	92.0	

Table E.8 Recovery data for multi-element-spiked feathers: nickel

Sample	Ni spiked (µg)	Spiked [Ni] in sample after treatment (µg/g)	[Ni] blank-corrected (µg/g)	Recovery (%)	Average recovery (%)
1	10	1333.3	961.7	72.1	
2	50	2193.0	1515.4	69.1	
3	100	6250.0	3656.9	58.5	**77.8**
4	250	8992.8	8937.9	99.4	
5	500	9881.4	8862.7	89.7	

Table E.9 Recovery data for multi-element-spiked feathers: selenium

Sample	Se spiked (µg)	Spiked [Se] in sample after treatment (µg/g)	[Se] blank-corrected (µg/g)	Recovery (%)	Average recovery (%)
1	2.5	333.3	208.550	62.6	
2	12.5	548.2	357.192	65.2	
3	25	1562.5	833.571	53.3	**69.3**
4	62.5	2248.2	1971.250	87.7	
5	125	2470.4	1922.477	77.8	

Table E.10 Recovery data for multi-element-spiked feathers: vanadium

Sample	V spiked (µg)	Spiked [V] in sample after treatment (µg/g)	[V] blank-corrected (µg/g)	Recovery (%)	Average recovery (%)
1	25	3333.3	2405.750	72.2	
2	125	5482.5	3843.033	70.1	
3	250	15625.0	9174.000	58.7	**77.7**
4	625	22482.0	22364.757	99.5	
5	1250	24703.6	21806.537	88.3	

Table E.11 Recovery data for multi-element-spiked feathers: zinc

Sample	Zn spiked (µg)	Spiked [Zn] in sample after treatment (µg/g)	[Zn] blank-corrected (µg/g)	Recovery (%)	Average recovery (%)
1	10	1333.3	769.3	57.7	
2	50	2193.0	1354.2	61.8	
3	100	6250.0	3286.9	52.6	**68.2**
4	250	8992.8	7818.6	86.9	
5	500	9881.4	8102.8	82.0	