University of Liège
Faculty of Sciences
Oceanology

Common guillemot *Uria aalge* stranding at the Belgian coast : an ecotoxicological evalutation.

Virginie Debacker

2000

University of Liège
Faculty of Sciences
Oceanology

Common guillemot *Uria aalge* stranding at the Belgian coast : an ecotoxicological evalutation.

Virginie Debacker

2000

Acknowledgements

At the term of this long study, I wish to address to my supervisor, Professor Jean-Marie Bouquegneau, my sincere thanks as he supported and welcome me inside his Laboratory of Oceanology. He followed every step of this work and made himself available at numerous occasions to discuss, comment and exchange ideas.

Dr. Claire Daemers - Lambert certainly is an important pillar of this work. I thank her sincerely for all the discussions, positive and pertinent remarks as well as thorough support received.

Professors Freddy Coignoul (Veterinary College, ULg) and Claude Joiris (Vrije Universiteit Brussel, VUB) are to be thanked for their relevant comments and interventions.

Special thanks are addressed to my colleague, the Veterinary Doctor Thierry Jauniaux who performed all the necropsies and sampling with an endless enthusiasm whatever the conditions. His remarks and comments often gave a different and useful approach of the problem.

My colleague, Dr. Ludo Holsbeek (Vrije Universiteit Brussel, VUB), offered me a precious support throughout this study, with numerous helpful and straight-to-the-point discussions and comments.

As part of a multidisciplinary study concrete help, information and advises have been received from a lot of my colleagues, in addition to their everyday's work which contribute to maintain this unique MARIN association :

- to my 'IN' colleagues who are in charge with the Belgian Beached Bird Surveys, beached carcasses deposits and biometric analysis (Dr. P. Meire, H. Offringa, J. Seys, J. Van Waeyenberg) ;
- to my colleagues who coordinate the group's actions in addition to organizing sample collection and dispatching (BMM/UGMM : Dr. T. Jacques, J. Haelters and IRSNB : W. Roggeman and J. Tavernier) ;
- to the Veterinary Doctor John Van Gompel for his useful comments;
- to the many unknown volunteers working in the rehabilitation centres of Oostende, Nieuwpoort, Blankenberge and Het Zwin.

In the framework of the MARIN group, I also wish to thank the French contacts, especially Bernard Cadiou (Bretagne Vivante, SEPNB) for his help providing the specimens during the Erika's oil spill.

Financial support of the MARIN group came from the 'Impulse Programme in Marine Sciences supported by the Belgian state - The Prime Minister's Services - Scientific, Technical and Cultural Affairs.' (MS/03/31, MS/12/032, MS/12/033, MN/DD/50, MN/DD/51, MN/DD/52 and MN/DD/53). In its early days, the MARIN group also received financial support from the 'European Commission' (13M/91/138, 1366-188901) and by The French Community of Belgium (n° 89/94-131).

To my friend Nathalie Antoine-Mathieu who first worked on this project in its early stage. She gave me a precious insight of the scientific work.

To my colleagues at the laboratory:

- Dr. Patrick Dauby who was always available for all these things that make a searcher's life easier;
- Sylvie Gobert-Boxho who kindly performed part of the presented analysis;
- Krishna Das for our multiple interesting bibliographic exchanges and remarks;
- Gilles Lepoint, Fabienne Nyssen and Frédéric Mineur for their support;
- to those who have left but certainly contributed to the general atmosphere (Laurent, Isabelle, Stéphane and Jean-Pierre).

To the students I worked with, Astrid Rutten and Laure-Sophie Schiettecatte who enthusiastically performed part of the presented results.

Such a work would not have been possible without the much appreciated technical support of both our First Chief Technicians, Renzo Biondo and Jean-Marie Théate. Although part of a different laboratory, First Chief Technician Nicole Gérardin also always offered an open door to help solving my technical troubles.

For their practical support at numerous occasions, I wish to express my thanks to Professor René Machiroux and Doctors Michel Frankignoulle and Alain Norro.

Sorting results using the statistical tool first emerged with the advises of Professor Johanne Detilleux (Genetic Department, Veterinary College) and Michel Hansenne (Psychology, Faculty of Sciences). I wish to particularly thank Laurence Seidel (Medical Computing and Biostatistics, Faculty of Sciences) who helped me with the final analysis and always made herself available to answer my numerous questions.

I am also indebted to the many librarians, first to Philippe Mottet and Joëlle Samedi from our documentary unit (UD Spring, Chemistry) but also to those many unknown people who kindly answered my requests.

To my friends and colleagues from the **C**entre d'**A**nalyse des **R**ésidus en **T**races and to their boss, Professor Edwin De Pauw (CART, Laboratoire de Spectrométrie de Masse, ULg) for their unconditional and practical support.

To researchers: Eric Parmentier, Alberto Borghese, Costa Frangoulis who kindly offered their time and help.

Although not familiar with the subject, Fernand and Monique Corin patiently read and made very precious comments and remarks which undoubtedly improved the manuscript.

To my sister Céline, who is also more at ease with other subjects but nevertheless offered me a precious help to finalize the manuscript.

To my family and friends.

Contents

General introduction

General introduction

The image of the marine ecosytem would not be complete without its seabird component. These upper trophic level vertebrates have evolved and conquered highly specialized ecological niches. Like numerous other species, they had to face the growing anthropogenic impact on marine ecosystems over the last decades. Our historical lack of understanding of the ecology has resulted in the release of large amounts of diverse pollutants into the environment (air, soil and water), with no knowledge of their detrimental effects, in particular their toxic effects, their stability and their tendency to bioaccumulate in living organisms. A recent recognition of the urgent need for a sustainable use of the environment has led many governments to establish long-term biomonitoring programmes examining the nature and consequences of pollution and general environmental changes on the different marine compartments. The Belgian federal authorities, as responsible for a country bordering the highly polluted North Sea, have launched such a global scheme. Part of it is dedicated to seabirds in terms of pelagic top predators likely to reflect environmental pollutant levels. The present multidisciplinary study fits in this global program.

Offering much more than shipping routes and transport, the North Sea is recognised as of major importance for seabirds, being inhabited by some ten million individuals for the most part of the year. Many species are present in numbers that represent substantial proportions of their world populations, although none are endemic (Dunnet *et al.*, 1990; Tasker & Becker, 1992; North Sea Task Force, 1993a,b). A large breeding population of more than 4 million seabirds, representing some 28 species, is localized at colonies along the North Sea coasts. In addition, shorebirds (wading birds and ducks), also find a sheltering area within the southern North Sea and feed on mud flat or other intertidal areas along the coast. In autumn, many birds disperse at sea, joined by visitors from northern and western waters, to spend the wintering season in the North Sea (Tasker & Becker, 1992; Carter *et al.*, 1993; Skov *et al.*, 1996).

This study focuses on the common guillemot (*Uria aalge*), a species presenting several advantages. Abundant in the northern North Sea all the year round, this pelagic top predator spreads into the southern North Sea during the wintering season, with peak numbers recorded from December to February, followed by a massive return back to the breeding colonies (Tasker *et al.*, 1987; Camphuysen & Leopold, 1994; Offringa *et al.*, 1996). As for many other species the wintering season corresponds to a sensitive period during which guillemots have to face combined environmental and anthropogenic stress factors, often leading to high mortality rates, particularly among the unexperienced juveniles during their first winter (Tuck, 1961 cited by Ydenberg, 1989). Among stranded species collected dead or moribund on the beaches between November to early March, guillemots rank first in Belgium (Kuyken, 1978; Sheridan & Palmart, 1988; Seys & Meire, 1992; Jauniaux *et al.*, 1993; 1996, 1998; Seys *et al.*, 2000 *in prep.*) as well as in other North Sea bordering countries (Camphuysen 1989, 1990a,b; Harris & Wanless, 1996).

Ecotoxicological studies examining 'the ecological and toxicological effects of chemical pollutants on populations, communities and ecosystems with the fate (transport, transformation and breakdown) of such pollutants in the environment', (Forbes & Forbes, 1994) have often relied on top predator, pelagic seabirds as sentinel organisms (Hutton, 1981; Muirhead & Furness, 1988; Lock *et al.*, 1992; Stewart *et al.*, 1994, 1996, 1999; Wenzel & Gabrielsen 1995; Wenzel & Adelung, 1996). However, most of these studies were carried on robust individuals, often shot at sea or at their breeding grounds, while the vast majority of the stranded guillemots at the Belgian coast present evidence of a debilitating process (Jauniaux *et al.*, 1996, 1998). In this context, toxicological investigations could only be relevant when treated within a multidisciplinary framework (**M**arine **A**nimal **R**esearch and **I**ntervention **N**etwork, MARIN), taking into account pathological and ecological data.

During the present work, we have envisaged the potential role of heavy metal contaminants as indirectly enhancing the guillemot's mortality during its wintering in the southern North Sea. Heavy metal levels were, in particular, examined in relation to the bird's general body condition as well as its main pathological symptoms observed at necropsy. Possible detoxifying processes were investigated through the analysis of metallothionein levels in the animal's tissues, which were also related to the guillemot's general body condition.

References

CAMPHUYSEN, C.J. (1989). Beached bird surveys in The Netherlands, 1915-1988. Seabird mortality in the southern North Sea since the early days of oil pollution. (Techn. Rapport Vogelbescherming 1) Werkgroep Noordzee, Amsterdam, 322 pp.

CAMPHUYSEN, C.J. (1990a). Massastranding van alken *Alca torda* op de Nederlandse kust, jan-mrt 1990: aantal, leeftijd en oliebesmeuring. Sula 4: 135-138.

CAMPHUYSEN, C.J. (1990b). Massastranding van Alk *Alca torda* en zeekoet *Uria aalge* op de Nederlandse kust, jan-feb 1990. Sula 4: 23-25.

CAMPHUYSEN, C.J. & LEOPOLD, M.F. (1994). Atlas of seabirds in the southern North Sea. IBN Research Report 94/6, NIOZ Report 1994-8, Institute for Forestry and Nature Research, Dutch Seabird Group and Netherlands Institute for Sea Research, Texel, 126 pp.

CARTER, I.C., WILLIAMS, J.M., WEBB, A., TASKER, M.L. (1993). Seabird concentrations in the North Sea: An atlas of vulnerability to surface pollutants. Joint Nature Conservation Committee, Aberdeen, 1-37.

DUNNET, G.M., FURNESS, R.W., TASKER, M.L., BECKER, P.H. (1990). Seabirds ecology in the North Sea. Netherlands Journal of Sea Research 26 (2-4):387-425.

FORBES, V.E. & FORBES, T.L. (1994). Ecotoxicology in theory and practice. Chapman & Hall, London, 247 pp.

HARRIS, M.P. & WANLESS, S. (1996). Differential responses of Guillemot *Uria aalge* and Shag *Phalacrocorax aristotelis* to a late winter wreck. Bird Study 43:220-230.

HUTTON, M. (1981). Accumulation of heavy metals and selenium in three seabird species from the United Kingdom. Environmental Pollution (A) 26: 129-145.

JAUNIAUX T., BROSENS L., DEBACKER, V., BOUQUEGNEAU J.M., COIGNOUL, F. (1993). Pathology of oiled seabirds at the Belgian coast. Scientific Symposium on the 1993 North Sea Quality Status Report.

JAUNIAUX T., BROSENS L., FARNIR F., MANTECA C., LOSSON B., TAVERNIER J., VINDEVOGEL H., COIGNOUL F. (1996). Mortalité des oiseaux marins lors de l'hiver 1992-1993 le long du littoral belge. Annales de Médecine Vétérinaire 140: 149-159.

JAUNIAUX T., BROSENS L., MEIRE, P., OFFRINGA, H., COIGNOUL, F. (1998). Pathological investigations on guillemots (*Uria aalge*) stranded on the Belgian coast during the winter 1993-94. The Veterinary Record 143: 387-390.

KUYKEN, E. (1978). Beached birds surveys in Belgium. Ibis 120: 122-123.

LOCK, J.W., THOMPSON, D.R., FURNESS, R.W., BARTLE, J.A. (1992). Metal concentrations in seabirds of the New Zealand region. Environmental Pollution 75: 289-300.

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.

NORTH SEA TASK FORCE REPORT (1993a). North Sea Subregion 4: assessment report (Netherlands, Belgium, France, Germany and United Kingdom). Oslo and Paris Commission (Olsen & Olsen, Eds), 195 pp.

NORTH SEA TASK FORCE REPORT (1993b). North Sea Subregion 3b: assessment report: United Kingdom. Oslo and Paris Commission (Olsen & Olsen, Eds), 154 pp.

OFFRINGA, H., SEYS, J., VAN DEN BOSSCHE, W., MEIRE, P. (1996). Seabirds on the Channel doormat. Le Gerfaut 86: 3-71.

SEYS, J. & MEIRE, P. (1992). Resultaten stookolieslachtoffer-tellingen langs de Vlaamse kust in de periode Januari-April 1992. Rapport Instituut voor Natuurbehoud A92.084, 18 pp.

SEYS, J., OFFRINGA, H., VAN WAEYENBERG, J., MEIRE, P., KUIJKEN, E. (2000). Characteristics of seabirds on the Channel doormat: analysis of Belgian beached bird surveys. *In preparation*.

SHERIDAN, R. & PALMART, L. (1988). Analyse de l'échouage et des causes de mortalité d'oiseaux marins récoltés sur la côte Belge entre avril 1986 et mars 1987. Aves 25 (3/4): 153-170.

SKOV, H., CHRISTENSEN, K.D., DURINCK, J. (1996). Trends in marine oil pollution in Denmark 1984-1995. Arbejdsrapport n° 75, 62 pp.

STEWART, F.M., THOMPSON, D.R., FURNESS, R.W., HARRISSON, N. (1994). Seasonal variation in heavy metal levels in tissues of common guillemot, *Uria aalge* from northwest Scotland. Archives of Environmental Contamination and Toxicology 27: 168-175.

STEWART, F.M., FURNESS, R.W., MONTEIRO, L.R. (1996). Relationships between heavy metal and metallothionein concentrations in Lesser Black-backed Gulls, *Larus fuscus*, and Cory's Shearwater, *Calonectris diomedea*. Archives of Environmental Contamination and Toxicology 30: 299-305.

STEWART, F.M., PHILLIPS, R.A., BARTLE, J.A., CRAIG, J., SHOOTER, D. (1999). Influence of phylogeny, diet, moult schedule and sex on heavy metal concentrations in New Zealand Procellariiformes. Marine Ecology Progress Series 178: 295-305.

TASKER, M. & BECKER, P.H. (1992). Influences of human activities on seabirds populations in the North Sea. Netherlands Journal of Aquatic Ecology 26 (1): 59-73.

TASKER, M.L., WEBB, A., HALL, A.J., PIENKOWSKI, M.W., LANGSLOW, D.R.

(1987). Seabirds in the North Sea. Final Report of Phase 2 of the Nature Conservancy Council Seabird at sea Project, Peterborough, 336 pp.

TUCK, L. (1961). The murres: their distribution, populations and biology - a study of the case *Uria*. Queen's printer, Ottawa, Canada.

WENZEL, C. & GABRIELSEN, G.W. (1995). Trace element accumulation in three seabird species from Hornøya, Norway. Archives of Environmental Contamination and Toxicology 29: 198-206.

WENZEL, C. & ADELUNG, D. (1996). The suitability of oiled guillemots (*Uria aalge*) as monitoring organisms for geographical comparisons of trace element contaminants. Archives of Environmental Contamination and Toxicology 31: 368-377.

YDENBERG, R.C. (1989). Growth-mortality trade-offs and the evolution of juvenile life histories in the Alcidae. Ecology 70 (5): 1494-1506.

Aim of the thesis

Aim of the thesis

The aim of the present work is to determine the potential relationships existing between the common guillemot's health status and its contaminants loading (Cu, Zn, Fe and Cd) through:

- the study of the heavy metal levels in the tissues;
- the study of the heavy metal speciation using metallothionein analysis;
- the investigation of the potential relationship existing between the heavy metal levels in the tissues and the body condition.

Chapter 1 presents the studied species (*Uria aalge*) in its North Sea habitat. Heavy metals and their impact on seabirds are also presented in this chapter with a special interest given to studies relating heavy metal contamination to the bird's body condition.

The results are then presented in different chapters:

Chapter 2: Ecotoxicological and pathological studies of common guillemots *Uria aalge*, beached on the Belgian coast during six succesive wintering periods (1989-90 to 1994-95).

In this study, guillemots collected directly on the beaches and those which had been treated in rehabilation centres were both analyzed. However, it soon appeared that, in addition to a general lack of information regarding the latter individuals, they were also characterized by significantly higher contamination levels. For these reasons, these birds were discarded from the sample and priority was given to those guillemots directly collected on the beaches.

In those beached birds, severe emaciation (cachectic status) was identified as one of the major lesion and linked to higher heavy metal levels (Cu, Zn and total Hg). At that stage, cachexia was evaluated as present or absent without intermediate status.

Compared to guillemots collected in more preserved area of the North Sea, those collected at the Belgian coast displayed higher heavy metal concentrations in their tissues (Cu, Zn and total Hg).

Chapter 3: Heavy metals contamination and body condition of wintering *guillemots (Uria aalge)* at the Belgian coast from 1993 to 1998.

In this study, the cachectic status is detailed depending on its severity (from non cachectic : '-' to low : '+1', moderate : '+2' and severely cachectic : '+3'). In addition, the general body condition of the guillemots is also evaluated using a condition index (liver to kidney weight ratio) which is clearly linked to the cachectic status: the lower the condition index, the more cachectic the bird.

Heavy metal levels (Cu and Zn) in the tissues were shown to significantly increase with increasing cachexia severity while the organs' total metal burdens remained unchanged. A general redistribution of heavy metals within the organs as a result of prolonged starvation and protein catabolism is proposed.

Chapter 4: Body condition and heavy metals : comparison between guillemots (*Uria aalge*) found stranded at the Belgian coast and those caught in the Erika's oil spill (Britanny, December 1999).

Comparing those two samples permitted :

- to examine the heavy metal contents in the tissues of healthy and robust guillemots. Results also pointed towards a probable general redistribution of heavy metals within the organs as a result of protein catabolism.

- to confirm that the southern North Sea, as a wintering ground for seabirds like the guillemots, is likely to be more polluted: significantly higher heavy metal levels were systematically found in guillemots collected at the Belgian coast compared to their Brittany counterparts, even when comparing both groups of more robust individuals (condition index >2.5).

Chapter 5: Combined effects of experimental heavy metals contamination (Cu, Zn and CH_3Hg) and starvation on quails' body condition: parallelism with a wild common guillemots (*Uria aalge*) population found stranded at the Belgian coast.

This experimental study using common quails was set up to evaluate the potential cause-effect relationship existing between the high heavy metal levels observed in the guillemots' tissues and their emaciation status: could these high levels induce emaciation or, on the contrary be the result of that emaciation process as previously suggested ?

Chapter 6: Influence of age, sex and body condition on Zn, Cu, Cd and metallothioneins in common guillemots (*Uria aalge*) stranded at the Belgian coast.

Heavy metal speciation was examined through the study of metallothionein, a metalloprotein known to play a key role in metal detoxification. Analysis were conducted on individuals for which the condition index ranged from low ($\leqslant 2$) to moderate (> 2) values, which also presented a wide range of total heavy metal levels in their tissues. The condition index appeared as significantly affecting the hepatic and renal metals distribution on the protein, with higher levels bound to the thionein in extremely emaciated guillemots.

Chapter 1

Literature study.

Contents

The common guillemot: *Uria aalge (Pontopiddan, 1763).*

Description of the species

Alcids (Charadriiformes) are wing-propelled diving seabirds which range from northern temperate to high arctic oceans. They are exclusively marine and represent 22 living species in 13 genera (appendix, Table 1), one species, the Great Auk (*Pinguinus impennis*) recently extinct (Storer, 1964 cited by Bédart, 1985; Cramp, 1985). The presence of almost all living and fossil specimens of *Alca* and *Alle* in the North Atlantic indicates that the Auks lineage, except *Uria*, evolved in the Atlantic Ocean (Moum *et al.*, 1994). According to Bédart (1985), *Uria* evolved in the Pacific Ocean after its ancestors found a route from the Atlantic to the Pacific Ocean before the opening of the Bering Detroit.

The common guillemot *Uria aalge*, (figure 1) in particular, is a boreal low arctic species breeding in the western north Atlantic and the largest living alcid (38 - 41 cm height, wing span 67-70 cm, body weight ± 1000 g.).

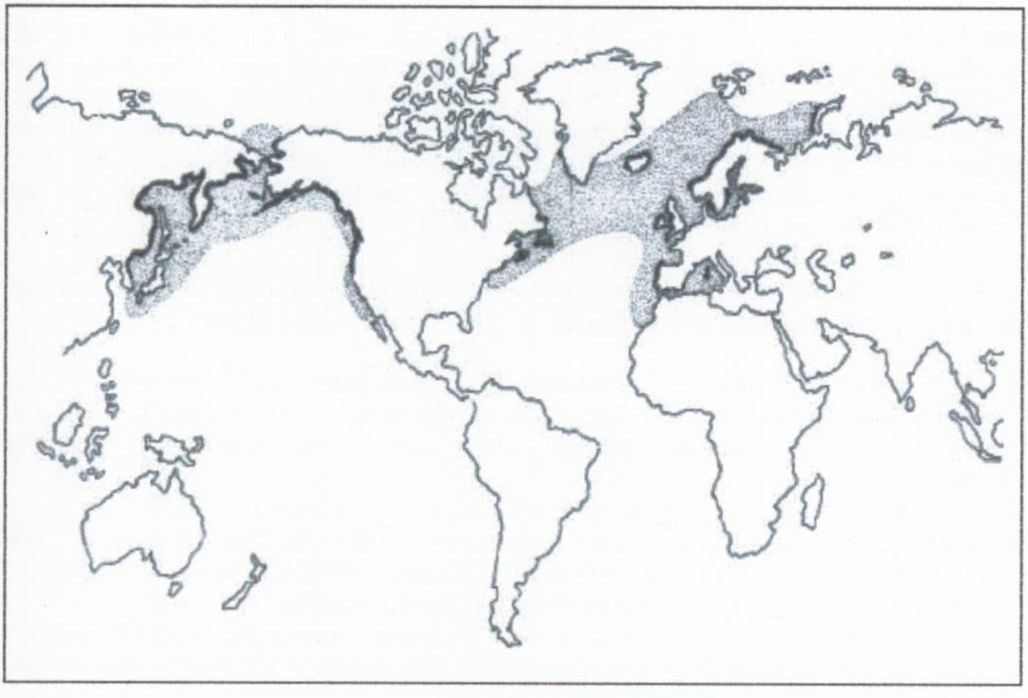


Figure 1: distribution of the common guillemot (*Uria aalge*) (from Freethy, R. 1987).

Although similar to penguins in being wing-propelled divers, though much smaller animals, alcids retained the ability to fly. They, nevertheless, are quite poor fliers and are much more efficient as divers. Several modifications in relation to underwater flight are apparent: shortening of the forearm, strong ligament structure of the wing with dense connective tissue, bones generally not pneumatic and relatively flexible, strong pectoral muscle and ability to reduce buoyancy when diving (Bédart, 1985).

Several subspecies of guillemots have been described (Salomonsen, 1944 and Storer, 1952 both cited by Bédart, 1985) within their range of distribution in the North Atlantic. However, all authors do not necessarily recognize these subspecies as little differences appear between some of them, a situation which still nourrishes a controverse. They recommended that the three most alike subspecies (*spiloptera*, *intermedia* and *aalge*) be regrouped under *aalge*. The two other forms, *albionis* and *hyperborea*, on the other hand, are considered as clearly recognizable (Bédart, 1985). The two main subspecies found at the Belgian coast are *Uria aalge albionis* and *Uria aalge aalge*, with *U.a. albionis* being more brownish-backed than *U.a.aalge* which is darker with a more blackish, slate-coloured back (Van Gompel, 1989; Seys & Meire, 1992).

Distribution in the North Sea

Though the majority of the colonies are localized around the British Isles and Scandinavia, a small and unique colony exists in the southeastern North sea on the Isle of Helgoland, Germany, where sandstone cliffs rising about 60 m above sea level provide breeding sites to guillemots but also fulmars (*Fulmarus glacialis*), kittiwakes (*Rissa tridactyla*), and razorbills (*Alca torda*). Numbers of guillemots at this colony have shown an increasing trend over the last decades, with an estimated population of around 2,500 pairs at the end of the 1980s (Leopold *et al.*, 1992). Counts of breeding guillemots around the British Isles also largely indicate an increase in number at the end of the 1980s (Nettleship & Evans, 1985; Furness, 1989; Tasker & Becker, 1992; North Sea Task Force, 1993b). On the contrary, auks, and guillemots in particular, are fairly uncommon in the Mediterranean Sea (Carboneras, 1988).

Common guillemots at the end of the breeding season tend to disperse in the North Sea, with no clear migration patterns. Nevertheless, some trends can be outlined (Brown, 1985):

- birds from Scottish and Faeroese colonies, and possibly also Iceland, move east to western Norway and in the North Sea. Young birds from southern Norway tend to move up the Norwegian coast during the winter while adults tend to move southwest into the western North Sea;
- some Scottish and Faeroese birds move westwards, towards Iceland;
- birds from Irish colonies either remain there or move south to the English Channel. Younger birds occasionally move further south and towards the southern North Sea;
- Helgoland birds mostly stay in the southern and central North Sea;
- guillemots from the south-central Baltic move to the south-west Baltic and the Kattegat;
- Barents Sea and northern Norwegian birds probably remain in the Barents Sea, although some spread southwest down the Norwegian coast.

Birds in their first and second year disperse more extensively than adult individuals (Birkhead, 1974). They usually come back to the colony for short visits during the breeding season and will progressively increase their stay on the breeding ledges as they grow older. As breeders, they show high site fidelity to their breeding colonies.

Ecological features

Feeding behaviour

Regarded as a stenophagous species, the common guillemot mainly feeds on pelagic fishes at all seasons of the year while molluscs, crustaceans, polychaetes and fish eggs represent only a small proportion of its diet (Belopol'skii, 1957; Braedstreet & Brown, 1985). Poor fliers, guillemots are anatomically designed for efficient underwater pursuit: hunting visually, they use their wings partly folded to propel themselves and swallow they prey at once during their dives (Bédart, 1969; Spring Lowell, 1971; Swennen & Duiven, 1977, 1991). Food digestion most probably occurs while at sea (Monaghan *et al.*, 1994; Gabrielsen, 1996).

Studies of dive speed, duration and depth show that guillemots can swim faster than their prey (2.2 ms^{-1} against 1.7 ms^{-1}, respectively) and at sometimes considerable depths (up to 180 m although 85 % of the dives were observed in the range 0 - 60 m depth) (Piatt & Nettleship, 1985; Swennen & Duiven, 1991). Wanless and co-authors, 1988 reported that guillemots can also perform relatively long dives (80 % of dives were of 20 -119 sec) with increasing pauses as the dive gets longer compared to other alcids like the puffin, *Fratercula arctica*, which make much shorter dives with very little pause duration (typically a few seconds) in between.

With such a foraging strategy, combined with their high latitude distribution, guillemots spend a considerable amount of time, at least half of their lives, at sea, on or under cold water masses. This cold aquatic environment poses a significant thermal challenge to these relatively small seabirds which mainly rely upon increasing metabolism to maintain body temperature (Gabrielsen *et al.*, 1988; Croll & McLaren, 1993).

Guillemots in the North Sea mainly rely on three fish families: Ammodytidae (sand eels: *Ammodytes marinus*), Clupeidae (herring: *Clupea harengus*, sprat: *Sprattus sprattus*) and Gadidae (cod: *Gadus morhua*, haddock: *Gadus aeglefinus*, whiting: *Merlangius merlangus*, saithe: *Pollachius virens,* Norway pout: *Trisopterus esmarkii*). Herring, sprat and sand eel are small schooling fish of high energetic values. In the North Atlantic, clupeids tend to be replaced by capelin (*Mallotus villosus*) (Montevecchi & Piatt, 1984; Tasker & Furness, 1996; Van Pelt *et al.*, 1997). Nevertheless, the guillemots also show an opportunistic behaviour to the extent of feeding on a variety of pelagic fishes. The relative proportion of the different species of fish eaten probably reflects their differing abundance in the feeding areas at the moment the guillemot feeds. Several studies demonstrated geographical variations of the importance of a pelagic fish prey in the bird's diet through the guillemot's distribution area but also from year to year at the same locality (Blake *et al.*, 1985; Barrett & Krasnov, 1996; Tasker & Furness, 1996; Lorentsen & Anker-Nielsen, 1999; Rowe *et al.*, 2000).

Seasonal changes also occur in the bird's diet, which has been widely studied during the breeding season but is less documented once the individuals disperse at sea during the wintering season. Invertebrates (polychaetes) are much more preyed upon during the winter in some areas while the birds shift from sand eels as main preys (spring to summer months) to clupeids and gadoids (September - January), with sand eels still taken but to a lesser extent (sand eels usually stay buried in bottom sediments during the winter) (Blake *et al*., 1985).

Guillemots, like other alcids, select their preys according to their diameter or body depth rather than their body length (Swennen & Duiven, 1991). They usually swallow smaller fishes than what their gape size would allow, with mean fish lengths ranging from 80 to 190 mm, with the majority in the range of 90 to 130 mm (Blake *et al*., 1985; Erikstad & Vader, 1989; Swennen & Duiven, 1991). Guillemots feeds on the juvenile stages of their main preys, although older age classes also appear in their diets during the summer breeding season (Wright & Tasker, 1996).

Studies of the total energy content (kJ) of the guillemot's prey indicates both a seasonal variation pattern and an intra-specific variation due to different age classes within one fish species, with increasing total energy content for older age classes compared to juveniles ones (Montevecchi & Piatt, 1984; Hislop *et al*., 1991; Van Pelt *et al*., 1997). Seasonal patterns are particularly clear for lesser sand eel (*Ammodytes marinus*) (Hislop *et al*., 1991) which, at a given length, approximately doubles its total energy content in summer compared to spring values. Sprats (*Sprattus sprattus*) (Montevecchi & Piatt, 1984) also exhibit temporal variations with highest energy contents in late fall and spring and lowest during the summer spawning season. Thus, when shifting from a diet mainly composed of sand eels in the summer to a diet which contains a higher proportion of sprat in the winter, the guillemot takes advantage of the temporal high energetic content of both of its preys. No seasonal changes were noticed for herring (*Clupea harengus*) or for whiting (*Merlangius merlangus*) (Hislop *et al*., 1991). Selection of prey based on its total energetic level is suggested by the authors and could undoubtedly be an important factor in the guillemot's feeding strategy during the breeding season. This could maximize its foraging effort regarding the fact that only a single fish is carried back to the young (Harris & Wanless, 1985). Furness & Barrett (1985) made similar observations at a colony in northern Norway where guillemots predominantly fed on gravid female capelin (higher lipid and protein contents) but could not demonstrate whether guillemots preyed selectively on the young ripe late-spawning capelins in the area or whether the behaviour of these capelins made them more available to the birds.

Daily food intake is about a quarter to a third of the adult body weight (950-1050 g) (Bédart, 1969). Captive common guillemots from the Murmansk coast were able to maintain their body weight with a daily intake of 300-350 g of fish (28.5 % of normal body weight), but died after 9 to 10 days, at 75 % of their normal weight, on a ration of only 100g. Birds given no food at all died after 6 to 8 days at 60 % of their normal weight (Golovkin, 1963 cited by Braedstreet and Brown, 1985). These figures are somehow lower than those reported in other studies. Cairns and co-workers (1990), using the doubly labelled water method to measure the metabolism, calculated a daily food intake of 511 g day^{-1} for breeding adults (Newfoundland, Canada) feeding mainly on capelin. Gabrielsen (1996) using the same method at the same period on Hornøya (Norway), reported a mean daily food intake of 440 g for adult birds feeding on capelins and sand eels, while a 15-day-old chick consumed around 105 g of fresh food per day. The author, however, underlines the fact that food composition, and more specifically its energy density, has an important effect on the calculation of daily food intake, thus leading to variable results (Monaghan *et al*., 1994; Gabrielsen, 1996).

Foraging activity

Foraging ranges are usually in close vicinity to the colony (5-10 kms) but can be much greater when prey abundance is low as birds will seek for better feeding grounds (Monaghan *et al.*, 1994). In particular, spatial summer distribution of its main preys clearly influences the guillemot's spatial distribution at sea during the breeding season, while it is less important during other times of the year probably due to changes in diet and prey availability (Wright & Begg, 1997). These preys are also more accessible to diving predators during the summer months, when they are widely distributed in the North Sea and active in the water column.

Food consumption is unevenly distributed across the North Sea, with areas of high consumption concentrated around breeding colonies (Furness, 1984; Tasker & Furness, 1996; Wanless *et al.*, 1998). Short-term feeding associations at sea have often been observed, between guillemots and/or other alcids (like the puffin or little auk) and other seabirds (kittiwakes, fulmars, gannets, sterns, gulls) but also with marine mammals (porpoises, dolphins, whales, seals). In this context, auks are seen as diving producers, apparently driving fish towards the surface. This joint feeding effort probably may facilitate seabirds to reach preys that would otherwise be unavailable to them (Evans, 1982, 1990; Skov *et al.*, 1995; Camphuysen, 1998; Camphuysen & Webb, 1999).

Outside the breeding season, guillemots have been shown to preferentially feed in areas where commercial sand eel fisheries catch rates are also high, suggesting common areas of exploitation (Wright & Begg, 1997). Some areas are of special concern, like fishing banks (*e.g.* Wee Bankie and Marr Bank, south-east Scotland) situated well within the feeding range of many seabirds breeding in colonies. Seabirds distribution at sea around these banks shows strong spatial overlap with the industrial sand eel fishery. Comparison of the size of the annual catch of the fishery and the amount taken by seabirds indicates that in most years the former has been consistently higher than the latter (Wanless *et al.*, 1998). Studies extended to the entire North Sea (Furness & Tasker, 1997) and over a whole year also concluded that the fishery was more likely to affect the seabirds than vice versa.

Drastic collapse in fish stocks, like those reported for capelins (*Mallotus villosus*) in the Gulf of Alaska and Barents Sea and for sprats (*Sprattus sprattus*) in the North Sea in the mid-80s were inevitably followed by signs of decline and changing in foraging behaviour of guillemot populations in the subsequent years (Harris & Bailey, 1992; Barrett & Krasnov, 1996; Van Pelt *et al.*, 1997). Immediate negative effects on adult's survival are unlikely as the birds can 'adjust' to a certain extent (shift to alternate preys or seek preys in other regions). On the contrary, local lack of preferred preys can have dramatic negative effects on seabird reproduction for breeding birds tied at the colony (Hunt *et al.*, 1996). Despite their apparent flexible time budget (flying to the foraging ground, dives duration, number of dives, time at the colony, rest time, …) (Burger & Piatt, 1990; Piatt, 1990; Monaghan *et al.*, 1996) the birds are unable to compensate fully for the reduction in food availability (Monaghan *et al.*, 1994) and although overall breeding success was not affected, provisioning rates were reduced and chicks were lighter in weight and took longer to fledge when food supplies were very low (Uttley *et al.*, 1994; Regehr & Rodway, 1999). Negative effects on chick's survival or growth rates were also noted for birds in large colonies compared to birds in smaller colonies, suggesting greater depletion of food around large colonies (Furness & Birkhead, 1984). According to Tasker and co-authors (1987) changes in the winter distribution of guillemots from the northeast Britain to the southern North Sea is likely to have followed a change in the distribution of sprat stock. A consequence of this southern shift is that the birds are spending the winter in much more polluted areas (contaminants, oil slick, heavy shipping routes..) and are thus more likely to be killed.

Reproductive strategy and breeding success

The guillemot, like other seabirds, has a delayed sexual maturity, the average age of first breeding being around 4.5 - 5 years old (Hudson, 1985). They usually breed in remote and inaccessible locations on cliffs and offshore islands which provide a breeding area safe from mammalian predators but not necessarily from avian predators (Harris & Birkhead, 1985; Ydenberg, 1989).

Occasional and irregular visits to the colony of adult breeders occur quite early in the season (at some colonies, sometimes as soon as october) but regular attendance at the colony usually happens one month prior egg-laying (Blake *et al.*, 1985; Harris & Birkhead, 1985). Non breeder individuals also come back at the colony for occasional visits which are observed increasingly with increasing age (Halley & Harris, 1993; Halley *et al.*, 1995). Breeding-site fidelity is extremely high (> 90 %) in adult birds which occupy and defend exactly the same spot from year to year (Birkhead, 1977; Harris & Birkhead, 1985; Harris & Wanless, 1988; Harris *et al.*, 1996).

Only one single pyriform egg (100-120 g) is laid and incubated during 30 to 40 days. Temporal variations of the successive breeding activities (laying, incubation, hatching, nestling, fledging) have been recorded at different colonies, with synchronicity being observed among members of the same colony (Birkhead, 1977; Harris & Birkhead, 1985). Nevertheless, individuals which breed later in the season are less likely to raise a chick successfully than those breeding earlier (Hedgren & Linnman, 1979).

Fishes are brought back one at a time to the chick by both parents but the chick is never left alone at the breeding site (Harris & Birkhead, 1985; Harris & Wanless, 1985). Sprats, sand eels, herrings and capelins are their main diet in various proportions due to prey availability and colony location (Braedstreet & Brown, 1985).

Young chicks, unable to fly, and their male parent leave the colony at the end of the breeding season while the female continues visiting the colony for up to two weeks after their departure. At fledging, the chick's mean weight (245-265 g) ranges between 20 to 27 % of the adult's mean weight, the rest of their growth being rapidly completed at sea (Hedgren & Linnman, 1979; Harris & Wanless, 1988; Ydenberg, 1989) where they reach about 90 - 95 % adult weight about 45 to 60 days after leaving the colony and become independent of the male about 25 days later (Varoujean *et al.*, 1979 cited by Harris and Birkhead, 1985). Many factors are likely to influence the breeding success (time of breeding, age of the female, quality of the breeding site, food availability…) which makes it difficult to compare the results from different colonies. Nevertheless, according to several studies at several breeding colonies, a pair of breeding guillemots produces about 0.62 - 0.82 young per year (Harris & Birkhead, 1985).

Survival rates

Estimates of immature survival to breeding age, compiled and presented by Hudson (1985), have been calculated following different techniques (1°: percentage of return of banded immatures to the breeding grounds. 2°: counts of band recoveries. 3°: comparison of chicks production in year 'N' with the proportion of 4 years old birds at the colony in year 'N + 4'.) and showed variations within one species (range from 17.4 - 41.1 % survival rate). This variation could be due to biases like emigration and intercolony movements of young guillemots but also to differences in techniques used. It could also be explained by geographical locations: figures of survival estimates tend to be greater in the western Atlantic than those in the eastern Atlantic, and within the eastern Atlantic, survival estimates are lower for colonies in southern Britain compared to northern colonies. Moreover, when considering postfledging mortality of juvenile alcids (calculated from Hudson's, 1985), Ydenberg (1989) shows that mortality is higher during the year immediately following fledging, when the chicks are smallest and least experienced (Tuck, 1961 cited by Ydenberg, 1989), and is not constant from year 1 to breeding age. Adult survival rates are shown to be over 94 %.

Moulting

Moulting occurs twice annually, with one complete moult just after the breeding season (mid-august) during which the bird is flightless and one partial pre-nuptial moult occuring straight after. The flightless period can last for about 45 to 50 days (Birkhead & Taylor, 1977; Bédart, 1985; Harris & Wanless, 1990). Timing of the pre-nuptial moult is different in adult, sub-adult (immature) and juvenile birds: most breeding adults moult to full summer plumage in November, immatures start moulting from January to March and most juveniles retain their winter plumage up until April (Camphuysen, 1995).

The North Sea

General presentation and characteristics

The North Sea is situated on the continental shelf of northwest Europe and is considered as one of the largest shallow-water areas of the world (figure 2). Semi-enclosed, it opens into the Atlantic Ocean to the north, via the English Channel to the southwest, and into the Baltic Sea to the east. Its neighbouring countries are: the United Kingdom, France, Belgium, The Netherlands, Germany, Denmark, Sweden and Norway. It covers a surface of about 750 000 km^2 and amounts to a volume of about 94 000 km^3 , which represents the studied area of the North Sea, divided into several subregions by '*The North Sea Task Force*' in the late 1980s early 1990s. The mean depth of the North Sea is 100 m and increases towards the Atlantic Ocean, with deepest areas north and west of the Shetland Islands (to about 200 m) and at the edge of the continental shelf. The Norwegian trench (± 725 m) extends from this deep water region around the coast of Norway and into the Skagerrak. The remainder of the North is less than 200 m in depth with shallower areas (<20 m) being found mainly off the coasts of continental Europe and southern England.

Figure 2 : map of the North Sea (Copyright Encarta Microsoft, 1996).

Both general oceanographic currents and tides and storms influence the hydrology movements within the North Sea. Water masses from the adjacent Atlantic, Baltic and Channel areas, each with their own characteristics, mix in the central North Sea. Coastal water masses, on the contrary, are largely influenced by fresh water inputs from the numerous rivers: the Elbe, the Weser, the Rhine, the Meuse, the Scheldt, the Seine, the Thames and the Humber contribute for up to 300 km^3 of freshwater each year. Several offshore areas, also characterized by shallow water, are known as banks (Dogger Bank, Fisher Bank, Turbot Bank, Smith Bank, Viking Bank…) and are of prime importance for fishing (Tasker *et al.*, 1987). Most of the seabed is made of sandy sediments and sandy gravel deposits with mud deposition in deeper areas. Rocks also occurs along the coast of Norway, Sweden and in the Waddenzee (North Sea Task Force Report 1993b).

The North Sea: an ecological approach

With highly industrialized areas in its bordering countries, the North Sea, from an environmental point of view, is often regarded as one of the most polluted seas in the world (Dunnet *et al.*, 1990; Laane, 1992; North Sea Task Force, 1993a,b). Several large rivers (Thames, Humber, Elbe, Weser, Meuse, Rhine, Scheldt, Seine) loaded with contaminants (both dissolved and on suspended particulate matter) continuously discharge into the North Sea where they disperse according to the currents or accumulate locally in sedimentation areas such as the intertidal zones and estuaries (Pedersen, 1996; Radach and Keyer, 1997). In addition to heavy anthropogenic inputs of pollutants through its main rivers, busy shipping routes and off-shore exploitations (gas and oil), the North Sea also provided waste incineration areas until early 1991, with subsequent high organochlorine residues levels in different benthic and pelagic organisms studied (Dethlesfen *et al.*, 1996). By far the largest industry in the North Sea, off-shore exploitations lands 92.5 10^9 m^3 of gas and 183 10^6 tons of oil per year, which are transported through a 10 000 km long pipe-lines network. Other activities, such as marine aggregate extraction contribute for up to 15 % of some bordering countries' demands for sand and gravel. Serious physical impacts are related to substratum removal and alteration of the bottom topography, such as the formation of temporary plumes in the water column, and re-deposition of material (de Groot, 1996). Re-suspension of contaminated sediments, like those occuring during dredging activities, especially in harbours and their vicinity, largely influences pollutants distribution in the water column, making them readily bioavailable to invertebrate organisms (Fichet *et al.*, 1998). A table showing the different range of heavy metal concentrations found in different compartments of the North Sea is given in the appendix (table 2).

Considerable efforts to understand the biogeochemistry of trace metals in the marine environment has been made over the last decades. Time trend analysis of pollutant inputs, and in particular heavy metals, developed with interest to determine historic additions and accumulation rates in coastal environments. Many studies either used sediments or fishes as chronological indicators and both methods underlined a recent decrease in heavy metal levels, especially lead (Jørgensen & Pedersen, 1994; Pedersen, 1996; Callaway *et al.*, 1998; Laane *et al.*, 1999). However, estuaries and their surroundings, receiving large input of metals from industrial sources, are still identified as areas where metal concentrations in sediments are clearly elevated. It has for instance, been estimated that between the Dutch-Belgian border and the open North Sea roughly half of Cd and almost 90 % of the PCB loads ends up in the sediment of the

Western Scheldt. Quantities of heavy metals directly discharged in the Belgian and Dutch coastal waters were, in 1990, estimated as follows: Cd: 1.8 ton/year; Pb: 20 tons/year; Hg: 0.4 ton/year; Zn: 180 tons/year and Cu: 28 tons/year. Offshore areas, on the contrary, are generally less contaminated, with levels not more than twice the concentrations typically found in open Atlantic waters (North Sea Task Force, 1993a,b; Plasman, 1998). Other studies, based on observation of malformation rates of fishes embryos in the North Sea also provide evidence of the deep impact of input of anthropogenic contaminants into the marine environment (Cameron & Von Westernhagen, 1997). Indications of changes in the marine flora and fauna of the North Sea due to habitat destruction, eutrophication, fishing and pollution have been documented over the last decades. A general loss of biodiversity in seaweeds and sea grasses as well as in benthic communities was observed, with a progressive but consistent shift towards opportunistic species (Ducrotoy, 1999; Frid & Hall, 1999; Lindeboom & de Groot, 1999; Philippart, 1998; Wolff, 2000a,b) .

Protection and management

Protection and management of the seas and oceans has greatly developed during the last 30 years. First concerned by the management of the fisheries resources and the prevention of oil pollution, international agreements soon progressed towards a global protection of the marine environment. In the North Sea, a first international agreement regarding the oil pollution was signed in the late 1960s (*Agreement for Co-operation in Dealing with Pollution of the North Sea by Oil*, Bonn, June 1969) and soon followed by the *Oslo Convention* (*Prevention of Marine Pollution by Dumping from Ships and Aircraft*, 1972) implementing regulations for dumping waste at sea. The next step was to prevent marine pollution from land-based sources, an agreement signed at Paris in 1974 (*Paris Convention for the Prevention of Marine Pollution by Landbased Sources*). Several international conferences then took place (Bremen, 1984; London, 1987; The Hague, 1990; Copenhagen, 1995; Lisbon, 1998) to co-ordinate, evaluate and re-adjust this international joint effort towards the protection, preservation and sustainable management of the North Sea environment (Tromp & Wieriks, 1994). All coastal states around the North Sea have ratified the *Ramsar Convention on Wetlands of International Importance*. Many of these wetlands (*i.e.*: the Flemish Banks situated within the Belgian area of interest, 51-52° N°), together with other sites, qualify as *Special Protection Areas* under the EC Directive on the Conservation of Wild Birds, 1979 (Carter *et al.*, 1993; Jacques, 1995).

Beached birds surveys in the North Sea: history and functions

Systematic monthly surveys of beaches have been widely used in different parts of the world to monitor the mortality of seabirds and in particular, mortality related to chronic oil pollution (reviewed by Burger & Fry, 1992 and Camphuysen *et al.*, 1999). This followed the discovery of oil fields at sea and concerns for seabirds vulnerability. Then, studies of their distribution patterns and movements for management purposes started. Little was know on seabirds distribution in the North Sea before the pioneer work of several different teams from different bordering countries. With internationally important seabirds breeding population within its territory, the United Kingdom (Britain, Ireland and adjacent islands) started to register important breeding sites by 1969-70 with '*Operation Seafarer*'. An almost complete database was achieved by mid-1980s (Furness, 1989; Lloyd *et al.*, 1991). Studies first largely focused on colonies during the 1970s and 1980s and then focused on seabirds offshore distribution and ecology. The *British Seabird Group* launched the '*Seabirds at Sea Project*' with ship-based and aerial surveys to provide information on the distribution of birds in inshore and offshore waters around UK (Tasker *et al.*, 1987). Ship-based surveys of seabirds (and usually also marine mammals) began in the 1970s in The Netherlands and were soon followed by aerial surveys in the mid 1980s (Camphuysen & Leopold, 1994).

Surveillance of illegal oil discharge and chronic oil pollution in the North Sea through counting of stranded oiled seabird carcasses also motivated the bordering countries to organize '*Beached Bird Surveys*' in the early 1970s. An international survey coordinated the effort of the participating countries until 1989: Norway, Denmark, Germany, The Netherlands, Belgium, France, Portugal and Britain. Thereafter, several national schemes were still carried out in different countries and many organised aerial flight surveillance as well in the 1990s (Kuyken, 1978; Camphuysen, 1989; Camphuysen & van Franeker, 1992; Skov *et al.*, 1996).

In Belgium, beached bird monitoring started in the late fifties with standardized surveys from 1962 onwards (Seys *et al.*, 2000 *in prep.*). Occasionnal land and ship based, but also aerial surveys, were conducted in the 1970s (Houwen, 1968; Joiris, 1972) as well as in other parts of the North Sea (Joiris, 1978; 1983a,b). More systematic surveys of the Belgian coastal zone (figure 3) and continental shelf (*Institute of Nature Conservation*) in the late 1980s and early 1990s followed, first funded by the World Wide Fund for Nature and later by the *Management Unit of the North Sea and the Scheldt-estuary Mathematical Model* (*MUMM, Belgian Ministry of Public Health and Environment*) (Offringa *et al.*, 1996).

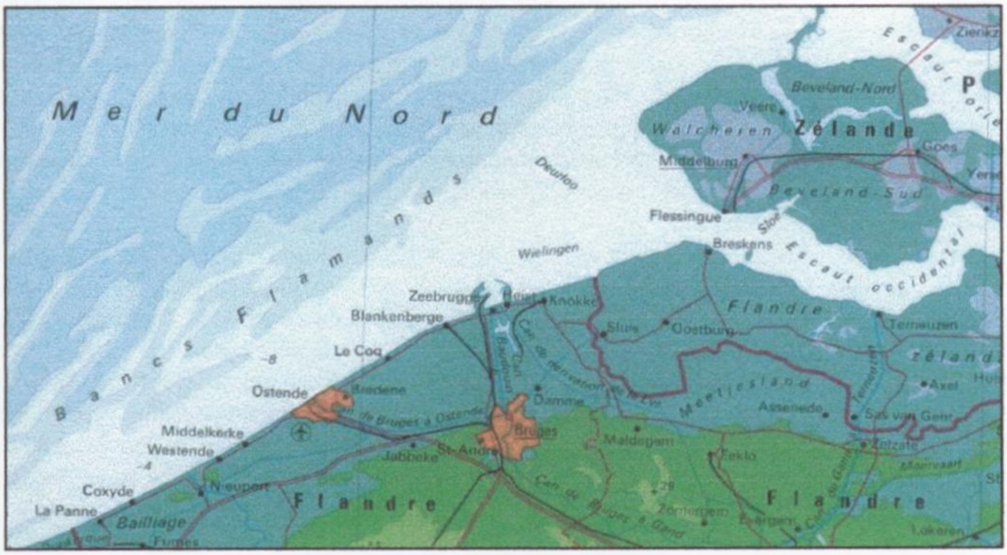


Figure 3 : map of the Belgian coast (Le Grand Atlas, De Boeck-Wesmael, Eds).

Unusual seabird mass stranding in the North Sea

Unusual mass stranding of dead or dying seabirds can have multiple explanations either related to natural (severe winter, storms, calm conditions, lack of food, toxins, diseases and parasites) and/or to artificial conditions such as large oil spill, chronic oil spills or chemical pollution. Vulnerability to wrecks is variable among species and is often linked to the bird's way of life: *e.g.* auks are known to be particularly vulnerable to oil pollution as are other species spending most of their time swimming (divers, ducks), while severe storms will affect flyers like storm petrels, fulmars or kittiwakes (Dorrestein & van der Hage, 1997; Camphuysen *et al.*, 1999). Guillemots is a predominant species among those found stranded at the Belgian coast (Kuyken, 1978; Sheridan & Palmart, 1988; Seys & Meire, 1992; Jauniaux *et al.*, 1993; 1996, 1998; Seys *et al.*, 2000 *in prep.*). Other typical species, but by far less numerous are razorbills (*Alca torda*), petrels (*Fulmarus glacialis*), herring gulls (*Larus argentatus*), kittiwakes (*Rissa tridactyla*) and oystercatchers (*Haematopus ostralegus*).

Oiling poses by far the greatest threat to seabirds in the North Sea (Carter *et al.*, 1993). Alcids are particularly vulnerable to surface pollutants and are often over-represented in large bird wrecks, not only in the North Sea but also in other marine areas (Stowe & Underwood, 1984; Camphuysen, 1989, 1998; Bodkin & Jameson, 1991; Harris & Wanless, 1996; Piatt & Ford, 1996; Skov *et al.*, 1996; Oka & Okuyama, 2000). Large numbers were associated to important shipwrecks (*Torrey Canyon*, 1967; *Amoco Cadiz*, 1978; *Erika*, 1999 in Brittany; *Braer*, in Shetlands, 1993; *Sea Empress*, in Wales, 1996 and the *Exxon Valdez*, 1989 in Alaska) but casualties due to chronic oil pollution are as numerous on a long term basis. Oil slicks at sea, rather than large oiling incidents on oil platforms or vessels are usually much more numerous, especially in the close vicinity of major shipping lanes in the North Sea (Camphuysen, 1989; Dahlmann *et al.*, 1994). Hence, the oil rate (ratio of oiled birds to the total stranded) observed for a particular seabird species, like the guillemot, clearly reflects differences in shipping intensity in the North Sea. For this species, higher oil rates are observed around the Channel, the Southern Bight and the Skagerrak regions, while comparatively lower oil rates are typical of the north, northwest and on Atlantic coasts (Furness & Camphuysen, 1997).

Parts of these hydrocarbon residues are eliminated at sea by natural processes like evaporation and bacterian oxydation, but these are by far overwhelmed by the frequency of such spills. International agreement to regulate discharge of oil from ships into the marine environment were taken through the MARPOL 73/78 convention. In 1985, upper threshold of legal discharges (discharge of oil above 30 litres per mile are illegal) as well as special areas in which any discharge are prohibited were defined. In the following decade, significant decline in chronic oil pollution in the southern North Sea seemed to indicate that positive effects of these legislation were obtained (Camphuysen, 1998). However, joint efforts through international surveillance of the beaches as well as cooperation of prosecuting authorities and more stringent controls of chemical tankers in harbours should not be diminished.

External contamination with oil alters the structure of feather barbules causing a loss of insulation, reduced buoyancy and water repellent properties. As a consequence of the plumage wetting and loss of insulation, the bird fails to maintain its body temperature (normally around

39°C) (Iversen & Krog, 1972) and consequently increases its metabolic rate to survive. Rapid heat loss in a cold environment, combined with diving and feeding (starvation) problems quickly lead the bird to death. For instance, in such conditions, oiled Rhinoceros auklets (*Cerorhinca monocerata* – Alcids) in the southern Japan Sea, were estimated to die within a day or two in cold weather (Oka & Okuyama, 2000). Once the bird is externally contaminated, ingestion of oil residues is not uncommon, following normal preening (as much as 7 ml oil/kg/day for experimentally oiled ducks) and cleaning activities. These residues act as poisons causing a variety of physiological and pathological alterations. Although the oils encountered in the wild can be of quite different composition (weathering), they may cause similar alterations, including overall weight loss, dehydration, hepatocellular dissociation, hemosiderosis, intestinal mucosal damages, adrenal or salt glands hypertrophy and renal tubular necrosis (Leighton *et al.*, 1983; Fry & Lowenstine, 1985; Peakall *et al.*, 1985; Khan & Ryan, 1991; Khan & Nag, 1993; Leighton, 1993). Indirect evidence of immunosuppressive effects of oil poisoning came from experimentation with mallard ducks (*Anas platyrhynchos*) and evidence from seabird rehabilitation centres. Leukocytes numbers (especially lymphocytes) were depressed in the major lymphoid organs (spleen and Bursa of Fabricius) and the peripherial blood. Secondary fungal and bacterial infections that contribute to morbidity and mortality among seabirds in rehabilitation centres also emphasize the immunosuppressive qualities of petrochemicals (reviewed by Briggs *et al.*, 1996).

Apart from mineral oils, unusual stranding due to other non-mineral oil, such as nonylphenol ($C_9H_{19}C_6H_4OH$), dodecylphenol ($C_{12}H_{25}C_6H_4OH$), bis-phenol ($HOC_6H_4C_6H_4OH$), vegetable oil, paraffin waxes… have been recorded. This was the case in The Netherlands (Zealand) during the wintering season of 1988-89 (Zoun *et al.*, 1991) and again in december 1998. Hundreds of casualties, among which numerous guillemots, were recovered all covered with a whitish, sticky substance, identified as polyisobutylene $(C_4H_8)_n$ (PIB). Surprisingly, severe lesions of the soft parts (bill, eyes, throat, feet web) were noticed at dissection on these glued birds while 'classically' oiled birds did not present any of these lesions. However, as PIB is recognized as non-aggressive substance, it was concluded that these dramatic soft parts disintegration could be linked to yet another chemical undetected during the analyses (Camphuysen *et al.*, 1998). Carcasses presenting identical characteristics were also recorded at the Belgian coast in 1998 (J. Seys, *pers. com.*)

When basic activities such as foraging and feeding are compromised due to low food availability or abundance, emaciated and exhausted birds rapidly wreck. Starvation, in combination with cold stress, was a major cause of large auks and kittiwakes wrecks described along the east coast of Britain in February 1983 (Blake, 1984; Hope Jones *et al.*, 1984) but also in the southern North Sea (Camphuysen, 1989, 1990a,b). Collapse of sprat (*Sprattus sprattus*) stocks in the Northern North Sea paralleled this wreck with birds shifting their wintering distribution east and southwards towards better feeding grounds. Similar dramatic wrecks occurred in Finmark during the winter of 1986/87, where thousands of emaciated guillemots were washed ashore after a rapid decline in Barents Sea capelin *Mallotus villosus* (Barrett & Krasnov, 1996).

Incidental entanglement in fixed gillnets is yet another threat to pursuit divers like the guillemot. In the North Sea, few studies report this kind of mortality (razorbills and guillemots killed by fixed salmon nets in northeast Scotland, studied by Murray and co-authors, 1994; the diet of guillemots entangled in cod nets set along the Norwegian coast, studied by Lorentsen & Anker-Nielsen, 1999). On the contrary, this phenomenom is well documented along the American coasts of California and Oregon as well as along Canadian shores (Piatt & Nettleship, 1987; DeGange *et al.*, 1993). Similar tools to those used to reduce marine mammals bycatch, *i.e.* visual and acoustic alerts, have recently been successfully applied to seabirds along the North American coasts (Melvin *et al.*, 1999). Fragments of fishing nets or lost drift nets, however, pose a serious entanglement hazard as well, particularly to gannets which often use net fragments and plastic waste as nest-building material. In the North Sea, ingestion of plastic debris was first documented in the early 1970s and usually involves small number of birds, like the Procellariiformes (petrel, fulmar) (Furness, 1985; van Franeker, 1985). Accumulation in the gizzard is typical with subsequent damages to the alimentary tract (reviewed by Dunnet *et al.*, 1990). Similarly, all sorts of plastic debris (small plastic spherules, condom and fishing line) were described by Jauniaux and co-workers (1996) in the gizzard of 1 guillemot, 3 fulmars and 1 herring gull collected stranded at the Belgian coast during the 1992-93 wintering season.

Mobile species like birds may be sequentially and/or chronically exposed to a wide variety of contaminants by external contact or ingestion (Furness, 1993; Nisbet, 1994). Pollutants like organochlorines and heavy metals, have been widely considered as potentially affecting the bird's fitness through increased reproductive dysfunction, changes in normal behaviour patterns, and increased susceptibility to diseases or other stresses (reviewed by Scheuhammer, 1987). Multidsciplinary ecotoxicological studies are viewed as a interesting tool to understand the influence of pollutants and their long-term effects on the marine environment (Luoma, 1996).

Heavy metals

The following presentation focus on Cu, Zn, Fe, and Cd as these are mainly discussed in the following chapters. Reference to seabirds is made for each trace element. An extensive body of literature exists relating to heavy metal analysis in seabirds, and discussing them in detail is beyond the scope of this introduction. These studies usually aimed at either:

- mapping heavy metal levels in a top predator compartment of a precise marine ecosystem;
- establishing geographical and temporal trends;
- studying the contaminants's distribution, accumulation and detoxifying processes within the tissues.

Results are usually discussed in terms of dietary, age, sex, intra- and inter-specific variations but also in relation to physiological factors such as moulting and breeding. Many address particularly toxic metals (Hg, Pb, Cd) with comparatively little informations on essential metals like Zn and Cu which are usually considered as homeostatically controlled. However, in the following, a particular attention is given to those studies referring to the animal's body condition in relation to heavy metal contamination.

General presentation and toxicity mechanisms

Metals, usually referred to as trace elements, are usually divided into essentials (Cu, Zn, Cr, Ni, Co, Ag…), required as trace (mmol/kg wet weight) and ultra trace (< µmol/kg wet weight) amounts by the living organisms, and non essentials (Cd, Pb, Hg…) with no known biological functions. Essentiality of a metal is established using two criteria:

1) the absence or deficiency of the element results in the impairment of life processes;

2) the impairment can be prevented or corrected only by the maintenance or addition of physiological levels of the metal and not by others.

In general, heavy metal ions are chemically very reactive, with a high affinity especially for sulfhydryl groups in proteins and smaller biological molecules. Enzymes, through binding metals at their active site, stabilize their secondary and tertiary structure and become active (Venugopal & Luckey, 1975). Both essential and non essential metals, due to their high reactivity with biological systems, can exhibit inhibitory and toxic effects, respectively at elevated and low concentrations (Mason & Jenkins, 1995). Metallic ions, unlike other toxicants which have well defined targets, are able to form either ionic or covalent complexes, with different affinities, with many electron donors atoms such as derivatives of nitrogen, sulphur and oxygen, in cellular components (Webb, 1977). Such interactions alter normal biological function and toxic metallic ions exert their toxicity with one of the following consequences:

1. blocking the essential functional groups of biomolecules;
2. displacing essential metal ions in biomolecules;
3. modifying the active conformation of biomolecules.

Protective detoxication mechanisms are found from primitive life stage such as prokaryotes to higher terrestrial and marine organisms.

Limiting metal accumulation :

- control of metal uptake;
- metal excretion;

Control of intracellular metal speciation :

- metallothioneins (see below);
- other low molecular weight ligands;

  e.g. glutathione, $C_{10}H_{17}O_6N_3S$ (GSH) through its thiolate sulfur atom can form GS-Me complexes with various metals, the rank of affinity for the -SH residue being Hg(II) > Cu(I) > Cd(II) > Cu(II) > Zn(II). GSH has thus been proposed as a first line of defence against heavy metal cytotoxicity, capable of detoxifying and complexing heavy metal cations soon after they enter the cells (Viarengo & Nott, 1993;Viarengo *et al.*, 1998; Canesi *et al.*, 1999);
- metal containing granules (membrane-limited vesicles such as lysosomes);

  These vesicles accumulate high concentrations of heavy metals in non toxic forms, probably bound to lipofuscin granules. Lipofuscins are mainly lipid peroxidation end-products which are accumulated in the lysosomes as insoluble lipoprotein granules and finally excreted by exocytosis. The metals are thus detoxified and finally excreted by the exocytosis of the lipofuscin granules. This process can be viewed as a general heavy metal homeostasis mechanism, potentially present in the cells of all living organisms. However, studies have demonstrated that this process is particularly active in marine invertebrate kidney cells which often have a lysosomial system rich in lipofuscin (Viarengo & Nott, 1993).

- formation of insoluble precipitate such as Ca/Mg concretions and Cu/S or Fe granules;

  This presumed detoxifying process is particularly prevalent in invertebrates although found in most of the major phyla. These granules, also termed concretions, storage vesicles, deposits, spherites, crystalloids, calcospherites, nephroliths, inclusions and bodies in the literature, all refer to metal-rich inclusions containing a high inorganic mineral content. They can be either intracellular or extracellular and are highly variable in forms. In invertebrates, although found in various tissues, they tend to be localized most commonly in the cells of the digestive and excretory epithelia (Mason & Jenkins, 1995).

Uptake and excretion

In higher organisms, the main routes of entry are (reviewed by Foulkes, 2000):

- intestinal mucosal cells;

  Both Ca and Zn transporters were described as potential carriers for toxic metal like Cd, however, the discussion is still open.

- alveolar epithelium cells;
- erythrocytes;

  These cells effectively accumulates toxic metals like Pb and Cd. The erythrocyte membrane possess a great anion permeability particularly sensitive to diisocyanatostilbene disulfonate (DIDS), a classical inhibitor of the anion channel. Uptake of a number of metals, including, Cu, Zn, Pb and Cd, is depressed by μM concentrations of this compound, suggesting that the metals can cross the membranes through the anion channel in the form of anionic complexes with carbonate, bicarbonate, hydroxyl, or chloride ions.

- hepatocytes;

  Several studies suggested that most of the Cd entering these cells use the Ca channels. However, the process still remains unclear.

- renal tubule cells.

  Non essential metals enters the tubule cells across both apical and basolateral membranes, often presented to the membranes under a complexed form with a low molecular weight protein like metallothionein, or other small molecules like cysteine and glutathione.

Transfer of heavy metals across cell membranes has been studied in numerous cell types, especially in the case of non essential metals like Cd and Hg. Despite many similarities, the mechanisms involved frequently exhibit properties characteristic of different cell lines, so that no one mechanism has been identified that could explain this process in all cells. However, due to their high reactivity for biological material, it appears unlikely that free metals, or partially bound metals (especially the non essential like Cd or Hg under its CH_3Hg form) can cross membranes without interacting with membrane constituents. Highly lipid soluble metal complexes (*e.g.*

dimethylmercury), on the other hand, rapidly and passively cross cell membranes and are extremely neurotoxic. Competition with existing transport systems designed to maintain homeostatic concentration of essential metals like Ca and Zn, exist in some cells but not in all cell types. In addition, physiological factors like the age can alter the characteristics of metal transport.

Heavy metals are mainly excreted via biliary secretions in the intestine and feces. Urinary excretion of both Cu and Zn is usually low (typically a few percent) but, however have been shown to increase under various conditions such as acute infectious diseases, stress and high protein metabolism. These metals are also common compounds of the sweat (Beisel *et al.*, 1976; Cousins, 1985; Elinder, 1986; Aaseth & Norseth, 1986). Due to its tendency to accumulate in the tissues, Cd is slowly excreted into the feces in a typical long-term low-level exposure. The fecal excretion comes mainly from the intestinal mucosa and only a smaller part originate from bile and pancreatic fluid. Significant increase of the excretion occurs when renal damages appear (Friberg *et al.*, 1986).

Copper

Uses and emission into the environment

Copper occurs naturally in many minerals either as a major component or in trace amounts in other minerals that make up the common rocks. It is probably the first metal worked by humans with first known artifacts of hammered Cu dated around 6000 BCE. Copper has largely been exploited ever since, with an increasing demand at the turn of this century as it is largely used in the electrical, construction, machinery, transportation (antifouling paints for boats), agriculture (insecticides, fungicides, algicides, repellents, fertilizers, preservative of woods, leather and other materials, veterinary products ...), chemicals and food industries (preservatives, additives, colouring agents).

In seawater and sediment interstitial waters, Cu^{+2} is the most readily available and toxic inorganic species of Cu, often found under the forms $Cu(OH)Cl$ and $Cu(OH)_2$, which account for about 65% of the total Cu in seawater. Trivalent Cu (Cu^{+3}) probably does not occur naturally (Boyle, 1979; Aaseth & Norseth, 1986 reviewed by Eisler, 1998). Bioavailability and toxicity of Cu in marine ecosystems is modified by several biological and abiotic variables, such as the presence of different acids (humic, fulvic, amino acid, ..) or the pH and salinity ranges. Copper residence time in deep ocean is evaluated as long as 1500 years compared to 1000 years in soils and only a few days (13 days) in the atmosphere. This latest compartment has a very small Cu burden compared to the others but is the most likely to be influenced by anthropogenic inputs. About 75 % of the total emission in the atmosphere results from human activities making this fraction readily available to be deposited in terrestrial ecosystems. Present anthropogenic inputs are two to five time higher than natural loadings (Nriagu, 1979).

Biological functions and toxicity

Copper is an essential element which is part of numerous enzymes catalyzing reactions known to be physiologically important in all living organisms, plants and animals (phenol oxidases, cytochrome oxidases, Cu/Zn superoxide dismutase, ceruloplasmin...). In animals, Cu is almost always tightly bound to proteins with very little occuring as the ionic -and thus toxic- species.

Liver is the key organ in the metabolism of copper which accumulates there when the excretory capacity of the hepatocyte is exceeded. Transport of Cu from the intestine to the liver occurs through binding to ligand(s), albumin being the major, but plasma Cu accumulates in the liver whether it is presented as amino acid complexes or as albumin-bound Cu to the hepatic tissue (Venugopal & Luckey, 1975; Cousins, 1985). Metabolic disorders may result both from Cu excess and deficiency. Copper is among the most toxic of the heavy metals in freshwater and marine biota, and often accumulates and causes irreversible harm to some species at concentrations just above levels required for growth and reproduction. The primary target for free cupric ions (Cu^{+2}) in the cellular membranes are thiol groups that reduce cupric to cuprous (Cu^{+1}) upon simultaneous oxidation to disulfides in the membrane. Cuprous ions are reoxidized to Cu^{2+} in the presence of molecular oxygen, which is thereby converted to the toxic superoxide radical (O^{-2}), inducing lipoperoxidation. Copper also interfer with normal enzyme activity and metallothionein (MT) synthesis (Aaseth & Norseth, 1986; Hall *et al.*, 1988 reviewed by Eisler, 1998). Recent studies presented by Fabisiak and co-workers (1999) demonstrated that sequestration of Cu on MT, under reducing condition, limits its ability to participate in a variety of redox cycling reactions which could give rise to reactive oxygen species and thus potentiate cellular damage.

High hepatic concentrations may be associated with liver damage, including hereditary conditions such as Wilson's disease in man and chronic copper poisoning in exposed livestocks or workers. In humans, normal dietary intake is 2 to 5 mg/day, toxic intake is > 250 mg, and total mass of the element in an average (70 kg) person is 73 mg (Pais & Benton Jones, 1997).

Cu and seabirds

The natural content of hepatic Cu of various vertebrate species may vary within a rather wide range. However, Cu, as essential element is often expected to be homeostatically controlled in the tissues with a relatively low intra-population variation (*e.g.* low coefficient of variation = 100 x STD/Mean) (reviewed by Thompson, 1990 and Walsh, 1990).

In wild birds, Norheim & Borch-Iohnsen (1990) described dense dark copper containing hepatic granules in the liver of eider (*Somateria mollissima*) with concentrations up to 1050 µg/g wet weight (± 263 ppm dw). The authors discuss these high Cu levels in terms of dietary intake as eiders mainly feed on invertebrates which rely on hemocyanin as a respiratory pigment. However, no signs of injury were observed.

Kim and co-authors (1998) reported 2 individuals of giant petrel (*Macronectes halli*) exhibiting hepatic Cu levels of 369 and 510 µg/g dw without apparent signs of toxic effects. The authors suggest a possible interaction between Ag and Cu as those 2 birds also displayed high hepatic Ag level and significant correlations were observed between these 2 elements. No mention of a possible seasonal accumulation pattern and/or a lessened body condition of these 2 specimens is made.

Zinc

Uses and emission in the environment

Zinc has been used by mankind for more than 2000 years, although its production largely increased at the end of the 19th century. Zinc ores (often mixed with Cd) are mined to a great extent (mainly in Canada, Russia and Japan) and substantial releases into the atmosphere occur during smelting activities. The metal is mainly used in the production of non-corrosive alloys, brass and galvanizing steel and iron products (used in the construction materials, automobile parts and household appliances). Zinc oxide is largely used has pigment while organic Zn compounds (carbamates) are used as pesticides (Elinder, 1986).

Biological functions

Zinc is an essential metal naturally present in all body cells of human (1.4 to 2.3 mg) providing essential structural and catalytic functions to a wide variety of proteins. More than 300 different enzymes depend on Zn for proper protein folding and biological function. Zinc is also crucial in the regulation of gene expression because numerous transcription factors have "zinc-finger motifs" that are maintaned by Zn. Highest concentrations are found in tissues such as liver, pancreas, kidney, adrenal glands, prostate and leukocytes where rapid deposition and turnover of this trace metal occur. Zinc naturally binds to metalloenzymes requiring Zn to exert their activity (*i.e*; carbonic anhydrase, carboxypeptidase, deshydrogenase lactique, alcohol deshydrogenase...). These metalloenzymes are present throughout all phyla and participate in a variety of metabolic processes including, glucose, lipid, protein and nucleic acid synthesis or degradation (Riordan & Vallee, 1976). Zinc is particularly important in tissues subject to active growth and metabolism, immune and inflammatory responses (macrophages and polymorphonuclear leukocytes activity), and repair following injury (Lansdown, 1995).

A growing body of evidence suggests a role for Zn in antioxidant defence systems. The metal has only one stable oxidation state (Zn^{2+}) and is not affected by free radicals or oxidative stress. It may act as a scavenger of free radical products through the synthesis of enzymes such as superoxide dismutase (SOD) and metallothionein or it may affect cytokine-activated transcription factors (Sturniolo *et al*., 2000). In human, daily dietary intake is 5 to 40 mg, toxic intake is 150 to 600 mg, lethal intake is 6 g, and total mass of the element in an average (70 kg) person is 2.3 g (Pais & Benton Jones, 1997).

Many common dietary components (*e.g.* phytic acid) can significantly reduce Zn absorption, resulting in Zn deficiency even with an adequate Zn content of the diet (Cousins, 1985; Pais & Benton Jones, 1997). Various factors have been documented to alter Zn homeostasis such as infection, starvation or inflammatory gastrointestinal diseases (Oberleas & Prasad, 1976).

Zinc and seabirds

Zinc as essential element is often expected to be homeostatically controlled in the tissues with a relatively low intra-population variation (*e.g.* low coefficient of variation = 100 x STD/Mean) (review by Thompson, 1990 and Walsh, 1990). Typical hepatic and renal Zn concentrations reported for different seabird species tend to be below 250 µg/g dw (reviewed by Thompson,

1990) although higher levels were reported in the northern fulmar (hepatic range: 225 to 688 µg/g dw), the Manx shearwater (pancreas range: 256 to 482 µg/g dw) (Osborn *et al.*, 1979), the great skua (hepatic range 61 to 497 µg/g dw) (Hutton, 1981), the black-footed and grey-headed albatrosses (hepatic range respectively of 150 to 398 and 28 to 296 µg/g dw) (Kim *et al.*, 1998). However, high hepatic and renal Zn levels are often found to significantly correlate with Cd levels, probably involving binding to metallothioneins (MTs) (Hutton, 1981; Nicholson *et al.*, 1983; Muirhead & Furness, 1988; Stewart *et al.*, 1997; Kim *et al.*, 1998).

Rattner & Jehl (1997) studying heavy metal loadings in relation to changes in body and organ masses in the Eared grebes (*Podiceps nigricollis*) demonstrated a clear redistribution of the body's Zn pool to the liver as a result of body weight loss. Mean hepatic Zn concentration were shown to increasse from 76 to 122 µg/g dw ($p< 0.05$) after a fasting period just prior to migrating to their wintering grounds. However, before fasting and migrating the grebes undergo a fattening period during which they increase their body and digestive organs weight. A higher dietary Zn intake is likely to occur during this period. A comparable situation is described for hepatic Fe in female eiders (*Somateria molissima*) which increase their dietary intake before starving and brooding (Borch-Iohnsen *et al.*, 1991). It is also notable that in both cases, both species increase their dietary intake prior to high consuming energy demands (respectively molting-migrating and egg laying-brooding).

A presumptive diagnosis of Zn toxicosis was made in four different species of diving ducks following ingestion of metal fence clips which contained 96 % of Zn. Clinical signs included weight loss, pectoral muscle atrophy, lethargy and anorexia (Zdziarski *et al.*, 1994).

Iron

Uses and emission in the environment

Iron is abundantly found in all compartment of the biosphere, from rocks, soils and water to all living organisms. Its geochemistry is complex and depends of its valence state as well as various physiochemical conditions such as pH.

Biological functions and toxicity

It is an essential metal of all living organisms, found in numerous proteins which act as electron transport ($Fe^{2+} \rightarrow Fe^{3+}$, cytochromes, ferredoxins), enzymes (catalase, peroxydase), or oxygen carriers (hemoglobin, myoglobin). Iron is absorbed through the intestinal tract and is transported via the portal vein to the liver but also to the spleen and bone marrow, bound to plasmatic proteins called transferrin. Within the hepatocyte, Fe is stored on ferritin, a large protein containing as much as 4,500 iron atoms (under the form $Fe\,(OH)_3$) organized in a cristalline web. In humans, ferritin represents 0.5 – 1.0 g mainly found in the liver, spleen and bone marrow. These organs usually carry 10 – 20 % of the total body Fe load, the largest pool being the hemoglobin. The body Fe is thus found in different compartments:

- the functional Fe present in amounts of 30-40 mg/kg body weight, includes hemoglobin, muscle myoglobin and heme and non-heme iron containing enzymes;

- the storage Fe accounts for 0-20 mg/kg of body weight and mainly consist of ferritin-bound Fe;
- the Fe bound to a transport system like transferrin, interfacing between storage and functional compartments.

When the storage compartment gets critically overloaded, the transport mechanism becomes fully saturated, with the excess Fe bound to non-transferrin compounds in circulation. This form of Fe is more readily accessible to the different organs and can lead to organ damages (Baynes, 1996). In humans, daily dietary intake is 6 to 40 mg, toxic intake is 200 mg, lethal intake is 7 to 35 g, and total mass of the element in an average (70 kg) person is 4.2 g. Iron deficiency anaemia is one of the ten most common human deficiencies (Pais & Benton Jones, 1997).

The liver is particularly susceptible to iron toxic effects. Primarily, Fe is located in the hepatocyte in a periportal to centrilobular gradient, but with increasing overload, Fe is found throughout the lobule, and also deposited in endothelial and Kupffer cells. Hepatic Fe overload leads to lipids peroxidation (via free radicals) of subcellular membranes with subsequent alterations of the functional properties of mitochondria, microsomes and lysosomes.

Fe and seabirds

Important accumulation of Fe in body tissues, especially the liver, is described for various species of wild and captive birds as haemosiderosis. Haemosiderin is an insoluble compound containing ferritin, proteins, lipids and degradation products and is found in lysosomes. Histological analyses of hepatic sections reveal the presence of this compound in hepatocytes and Kupffer cells but also in macrophage cells of the spleen. This excess stainable Fe in the liver is probably the result of an altered Fe metabolism with increased turnover of tissue Fe. Although hepatic haemosiderosis has been reported in birds without apparent liver disease, Fe overload is often associated with an overall lessened body condition following infectious diseases or starvation (Borch-Iohnsen *et al.*, 1991; Cork *et al.*, 1994; Dierenfeld *et al.*, 1994; Cork, 2000).

However, in wild migratory species, seasonal changes in tissue Fe occur as part of the normal physiological cycle and are associated with the breeding season and moult (Osborn, 1979; Honda *et al.*, 1986). Tolerance to iron overload is also likely to vary in individual species of birds, even within closely related taxonomic groups (Ward *et al.*, 1988; Cork, 2000).

Cadmium

Uses and emission in the environment

Cadmium is naturally present in the Earth's crust at very low concentrations (< 0.5 ppm), often associated with Zn in mixed ores. Increasing use and production of Cd (Ni/Cd batteries, sewage sludges, electroplating, coloured pigments, stabilizers for PVC, fungicides), especially after World War II, made it readily available to contaminate all compartments of the ecosystem even in remote areas where anthropognic pressure is relatively low.

Atmospheric deposition is of minor importance compared to those of Hg and Pb, because Cd is less volatile than these metals. However, riverine transport is more important and can lead to much higher soil concentrations. Riverine transport to marine habitats, with deposition on the bottom sediment of the continental shelf highly contributes to increasing levels in seas and oceans (Furness, 1996). In addition, acidification of freshwaters will increase the concentration of disssolved Cd, resulting in greater amounts of Cd being available for incorporation into the food chain (Scheuhammer, 1991). Typical depth distribution of Cd in oceans show that surface waters are depleted (± 0.01 µg/kg seawater) relative to the deeper ocean water (± 0.07 µg/kg), indicating uptake at the surface by biogenous materials (organisms and suspended particulate matter) and regeneration from sinking biologic debris deeper in the water column (Förstner, 1980). Cadmium is generally regarded as one of the most mobile toxic metals, because its transfer occurs among most terrestrial food chains (Peterson & Alloway, 1979; Furness, 1996).

Toxicity

Cadmium is an element with no known biological function which has an evident affinity for biogenous particulate material (Burton & Statham, 1990). Cadmium can affect many cellular functions and is considered as toxic even at low concentrations. Its toxicity and chronic effects on exposed workers were fully recognized after chronic Cd poisonning of Japanese populations known as the " Itaï-Itaï " disease (skeletal deformities), which occured in the early 1950s, killing many people (Piscator, 1976; Förstner, 1980). Upon uptake from digestive and/or respiratory tracts, Cd binds to albumin and other high molecular weight proteins, and then is taken up by the liver. Cadmium toxicity can be modified by a range of nutritional and physiological factors (*e.g.* interfer with essential element such as Ca, Zn, Cu and Fe metabolisms) (Bremmer, 1979).

In the liver, Cd accumulates through binding to a low molecular weight protein, metallothionein (MTs), which synthesis can be induced by the metal (Scheuhammer, 1987; Lauwerys, 1990; Beyersmann & Hechtenberg, 1997; Haschek & Rousseau, 1998). Cadmium bound to MTs (Cd-MT) is gradually released from the liver into blood plasma and then can easily reach the kidneys. Due to its small molecular weight, Cd-MT can pass through the glomerular membrane of the kidneys and is taken up by the renal tubules. In the blood, this metal is found within the erythrocytes bound to the hemoglobin and MTs. It is slowly excreted via biliary secretions and urine, due to its long biological half life (>15 years) and is thus easily accumulated in biological tissues such as liver, kidneys, testes, salivary and thyroïd glands. More than 50 % of the body total burden are found within the liver and kidneys (Lauwerys, 1990). In humans, daily dietary intake vary between 7 µg to 3 mg, toxic intake is 30 to 330 mg, lethal intake is 1.5 to 9 g, and total mass of the element in an average (70 kg) person is 50 mg (Pais & Benton Jones, 1997).

Most of the toxic effects of Cd are attributed to the intracellular reactions of the Cd^{+2} ions. It is generally assumed that the free hydrated metal ion Cd^{+2} is the bioavailable and toxic species for aquatic organisms. At elevated concentrations, Cd inhibits the synthesis of RNA, DNA, and proteins and induces lipid peroxidation, DNA strand breaks, and chromosomes aberrations. At noncytotoxic doses, it interferes with DNA repair processes and enhances the genotoxicity of directly acting mutagens (Beyersmann & Hechtenberg, 1997). Cadmium and its compounds have been recognized as carcinogenic to humans by the **I**nternational **A**gency for **R**esearch on **C**ancer (IARC, 1993).

Kidneys are particularly sensitive to cadmium toxicity as well as other heavy metals like Hg and Pb. Cadmium nephrotoxicity induces necrosis of the proximal tubular cells (*pars recta*), increases proteins excretion (proteinuria), increases glucose excretion (glycosuria) and increases Cd urinary excretion. It has been proposed that nephrotoxicity produced by chronic Cd exposure occurs via the Cd-MT complex and not by inorganic forms of Cd. However, Klaassen and co-workers (1999b), testing this hypothesis, used a MTs-null mice model demonstrated that although these animals could not form Cd-MT, they nevertheless developed nephrotoxicity to inorganic Cd. The authors conclude that Cd induced renal injury is not necessarily mediated through Cd-MT complex and that induction of MTs is an important adaptative mechanism protecting against chronic Cd nephrotoxicity. Other studies propose that the mechanism of Cd^{2+} effect on the proximal tubular cells involves free radical damage (Diamond & Zalups, 1998). These lesions are thought to develop once the MTs' capacity to bind Cd is overwhelmed and that free Cd ions can exert their toxicity. In man, such lesions were observed in workers whose Cd kidney cortex concentrations averaged 200 ppm wet weight (Piscator, 1976; Lauwerys, 1990). Testes are also severely affected.

Cd and seabirds

In wild birds, Cd concentrations vary widely among species and among population within species, with mean levels of < 0.1 to 32 mg/kg wet weight in the liver and < 0.3 to 137 mg/kg wet weight in the kidneys (Walsh, 1990). These variations are often attributed to dietary differences and/or seasonal variations as well as the influence of physiological parameters such as breeding and moult. Compared to land-based birds, seabirds typically display higher Cd concentrations, and among seabirds, highest levels have been observed for squid- and insect-eating species (Scheuhammer, 1987; Muirhead & Furness, 1988; Lock *et al.*, 1992; Stewart *et al.*, 1997, 1999).

Many studies have also underlined a general trend towards Cd accumulation with age in several seabird species, with highest concentrations found in adult tissues compared to juveniles (Thompson, 1990; Lock *et al.*, 1992; Stewart *et al.*, 1994, 1996; Wenzel & Gabrielsen, 1995; Wenzel & Adelung, 1996). However, as noted by Stewart & Furness (1998) very few studies have attempted to consider the possible continued accumulation of Cd with increasing adult age. According to their studies on several species (Lesser black-backed gulls, banded great skuas and adult Cory's shearwaters), the lack of evidence for continued accumulation throughout the bird's lifespan could be explained by a possible mechanism of Cd regulation. As described for marine mammals (reviewed by Das *et al.*, 1999, *in press*), seabirds, in response to the relatively high natural levels of Cd encountered in the marine environment through their evolution, may have developed a mechanism to cope with and/or regulate tissue concentrations of this metal. In some cases, significant positive correlations have been found between Se and Cd levels suggesting a potential detoxifying process of Cd through binding to Se (Furness & Hutton, 1979; Kim *et al.*, 1998).

Furness (1996) suggested treshold tissue concentrations for liver and kidneys, respectively of 40 and 100 ppm ww (± 160 and 400 ppm dw), above which cadmium poisoning might be expected. In birds, as in humans, Cd accumulates preferentially in the kidneys, with very low or undetectable levels in feathers and eggs (Scheuhammer, 1987; Thompson, 1990). Nicholson & Osborn (1983) and Nicholson and co-workers (1983) reported numerous lesions to renal tissue at different structure levels in the puffin (*Fratercula arctica*) and other pelagic seabirds (petrel, *Fulmarus glacialis* and Manx shearwater, *Puffinus puffinus*) displaying high Cd concentrations. These included damages to the glomeruli and proximal tubules but also ultrastructural changes in the distal tubules and collecting ducts of heavily loaded individuals (range of concentration: 30 to 250 ppm dw). At the cell level, degenerative processes were observed involving vacuolation of the cytoplasm, swollen mitochondria, numerous lysosomes, pynocytotic vesicles and coarsely granulated cytoplasm. Similar changes were also present in experimentally dosed starlings (*Sturnus vulgaris*) supporting the view that the seabird kidneys lesions were metal-induced and not a result of some other factors. However, these results are controversial as the birds studied by Nicholson & Osborn (1983) displayed renal Cd concentrations below those reported to affect kidneys structure in experimental studies using Cd alone, but they contained rather high concentrations of Hg that may have contributed substantially to the kidneys damages (Furness, 1996). For a similar range of concentrations reported for numerous species from the New Zealand region, Lock an co-workers (1992) did not observed any kidneys damages. Questions on the potential effects of these high levels on the individual's fitness are still to be clarified.

Other trace elements

Both teams of Forrester and co-workers (1997) and Daoust and co-workers (1998) correlating pathological and toxicological findings in common loons (*Gavia immer*) found stranded respectively in Florida and in Canada, observed significantly higher Pb, Se and Hg levels in loons in poor body condition (emaciated to varying degree, absence of sub-cutaneous and abdominal fat) compared to more robust specimens. Some loons display Pb concentrations compatible with poisoning (± 23 ppm dw). In parallel, the authors showed that significantly more parasites (trematodes) were found in birds with a low body condition which may result of a depressed immune function.

Metallothioneins

General structure and characteristics

Metallothioneins (MTs) are small cysteine-rich heavy metals binding proteins first discovered by Margoshes & Vallee (1957) in the horse kidneys. They differ from most other metalloproteins by their much higher metal content, their unusual structure and their remarkable ability to rapidly exchange metal ions. High affinity for heavy metals (Cu, Zn, Cd, Hg, Co, Ni, Au, Ag, Pb) is primarily due to numerous cysteine residues, often found as Cys-X-Cys (X being any amino acid) or Cys-Cys. These are also important for the structure of the protein through the formation of S-S bridges although, in MTs, cystein residues exist in a reduced state. A typical mammalian MT has a molecular weight of 6000-7000 Da, contains approximately 61 amino acids among which 25-30 % are cysteine residues and has a metal-binding capacity of between 7 to 10 g atoms/mol. Moreover, cysteine residues are invariably found at the same location along the amino acid sequence. Another feature of this protein is its lack of aromatic amino acid (tryptophan, tyrosin, phenylalanin). Spectroscopic and chemical studies of the protein revealed the existence of two metal-binding site or metal-thiolate clusters, one with 3 metals ions bound to 9 cysteines and the other with 4 metal ions bound to 11 cysteines (figure 4). All metals are tetrahedrally bound to four cysteine thiolate ligands.

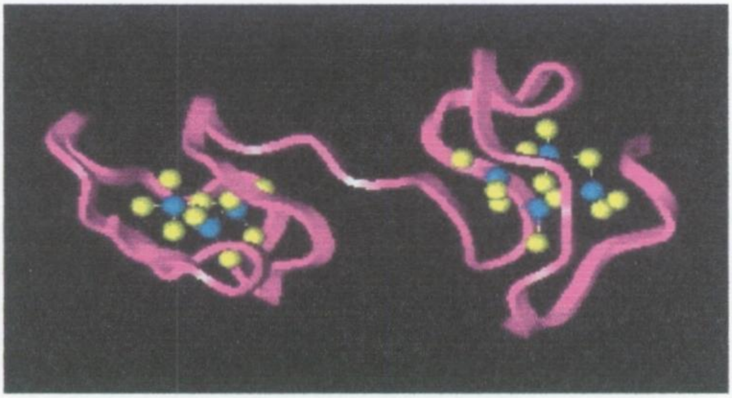


Figure 4: spatial structure of a rat MTs determined from 2D-NMR spectroscopy and X-ray crystallography showing the N-terminal *β* domain (3 metal cluster, blue points) and the C-terminal *α* domain (4 metal cluster, blue points). Kindly received from Dr. P-A. Binz.

These two clusters were later identified as two separate protein domain, the N-terminal *β* domain (3 metal cluster) and the C-terminal *α* domain (4 metal cluster). Metal binding in each cluster is cooperative, with initial binding to the four-metal cluster. Once it is saturated, binding occurs at the three-metal cluster. Release of metal is also cooperative, with metal leaving the three-metal cluster first. Different metals can be found bound to the same polypeptide depending upon which organism the MT was purified. For instance, human MT typically binds 7 atoms of either Zn, Cd or Hg and 12 atoms of Cu. It was deduced, knowing the number of cystein residues that Zn, Cd and Hg were bound in as divalent ions ($^{+2}$) and Cu as a monovalent form.

The binding stability follows the trend: $Hg^{2+} > Cu^{1+} > Cd^{2+} > Zn^{2+}$. Under normal physiological conditions Zn will be the primary metal bound to MT, the dissociation constant for this metal being 5×10^{-13} M at pH 7. This means that at picomolar concentrations of Zn, MTs are already highly saturated with Zn. This metal can, however, be readily displaced by Cu or Cd (Cousins, 1985; Klaassen *et al.*, 1999a).

Metallothioneins are widespread from prokaryotes, protists, fungi, plants, invertebrates to higher aquatic and terrestrial vertebrates. Primary structure of the protein has been widely studied in animals although similar structures also occur in plants, fungi and bacteria. In animals, MTs are primarily found in target organs for heavy metals accumulation, such as liver and kidneys but also in various tissues including the intestine, spleen, pancreas, brain, heart, testes and placenta. It is located mainly in the cell cytosol but can also occurs in the nucleus. The size of the protein as well as the amino acid composition can be very different depending on the species studied. The presence of different isoforms within a species has also been demonstrated. Two major isoforms are ususally found within mammalian tissues, MT-I and MT-II, slightly different in amino acid composition and separable by high performance liquid chromatography. Two additional isoforms of MTs, termed MT-III and MT-IV also occur in mammals and seems to be expressed under specific cell conditions. MT-III is expressed predominantly in neurons and glial cells but also in male reproductive organs. MT-IV is expressed in differentiating stratified squamous epithelial cells. However, MT-III and MT-IV are structurally similar to MT-I and MT-II, particularly in the placement of the 20 cysteine residues (Andrews *et al.*, 1996). Each class of isoform consists of several different isoproteins distinguished on their chromatographic behaviour. Mammalian class I and II MTs are single chain proteins containing 61 to 62 amino acid residues. Larger chains with 72 and 74 residues are found in mollusks and in nematodes respectively. Shorter chains occurs in insects and certain fungi, the shortest one, with 25 residues, in *Neurospora crassa* (Cousins, 1985; Hamer, 1986; Kägi, 1991). Analysis of the MT structure of different birds species (pheasant, quail, turkey and chicken) reveals a cysteine-rich protein that shares extensive structural homology with the mammalian MT with identical major amino acid sequence in each of these birds (Andrews *et al.*, 1996). The human genome contains 14 metallothionein genes, all containing the four isoforms, located on chromosome 16. Six of these are functional, two are not and the remaining six have not yet been characterized. These genes display complex promoters containing multiple metalloregulatory elements (MREs). These MREs within one promoter are not identical and do not respond uniformly to different MRE binding proteins, the response being either positive (activation) or negative (inhibition) of MT transcription.

MT synthesis can be induced by a variety of agents and conditions (table 1) among which heavy metals rank first, especially Cd, Cu and Zn. However, Jacob and co-workers (1999) working on the mouse suggest that different factors can operate in response to different inducers that lead to activation of the same gene promoter, namely the MT gene. The authors showed that during restraint stress, MTs are induced as a result of activation of glucocorticoid receptor (glucorticoid response element, GRE) whereas the heavy metals mediate this process via activation of other factors such as metal transcription factor (*e.g.* MTF-1).

Agents and condition inducing metallothioneins synthesis.
Metal ions: Cd, Zn, Cu, Hg, Au, Ag, Co, Ni, Bi
Hormones: glucocorticoïdes, estrogen, progesterone
Cellular second messengers: glucagon, angiotensin, cathecholamin, cAMP, …
Growth factors: insulin, IGF, EGF, …
Inflammatory agents: interleukins, endotoxin, …
Antibiotics: cycloheximide, mitomycin, streptozotocin, …
Cytotoxic agents: ethanol, isopropanol, chloroform, bromo-benzene, carbon tetrachloride,…
Vitamins: ascorbic acid, retinoate, vitamin D_3,…
Stress producing conditions: starvation, infection, inflammatory, physical stress.

Table 1: agents and condition inducing MTs synthesis (Kägi, 1991, 1993).

Despite this well defined structure, MTs are considered as relatively flexible proteins, capable of fixing different metal ions having different ionic radius, with constant metal exchange within and/or between the clusters. In addition, MTs can donate Zn to enzymes requiring Zn in their active site for adequate activity. Recent studies further confirm that the exchange rate of metals, like Zn, between MTs and Zn requiring proteins is far more efficient than between free Zn in solution and the protein alone (Maret *et al.*, 1997; Jiang *et al.*, 1998). These findings also support the widespread opinion considering that the fundamental role of MTs is to control the homeostasis of essential trace elements, such as Zn and Cu (Hamer, 1986). Maret & Vallee (1998) and Fabisiak *et al.* (1999) further suggest that the redox state of the cell could be conditioning MTs control of metals homeostasis, with enhanced release of metals (like Cu and Zn) under oxidative cell conditions. It appears that MTs containing Zn and Cu functions as an antioxidant.

In addition, MTs are strongly believed to act in the detoxification of non essential trace elements such as Cd and Hg (Hamer, 1986; Kägi, 1991, 1993; Roesijadi, 1992, 1996). Mutation that prevent MTs synthesis confer metal sensitivity, whereas excess expression of MTs enhance resistance to metals toxicity. Genetically modified MT-null mice (MTs -/-), unable to transcript the MTs I and II genes are far more sensitive to Cd toxicity compared to the wild-type mouse (MT +/+). Sensitivity to DNA damaging agents leading to cell death (apoptosis) has also been demonstrated for these MTs-null mice. Such results also implicates MTs synthesis as stress-inducible (Kondo *et al.*, 1997; Klaassen & Liu, 1998; Jacob *et al.*, 1999).

Reduced food intake, as well as protein deficient diets, are known to result in increased MTs gene expression in animals. The exact mechanism is still unclear. This may be due to the influence of elevated levels of glucorticoids and catecholamine hormones, which are part of the physiological response to starvation in animals. Different studies suggest that MTs biosynthesis could be part of a general response for the adaptation of organisms to stressful conditions and cell damages. It has been thus proposed that MTs could function as a scavenger of oxygen free radicals produced during physiological stress (Hidalgo *et al.*, 1990; Bremmer, 1991; Viarengo *et al.*, 1998).

High levels of MTs have also been detected in the nucleus and in the cytoplasm in both begnin and malignant tumors (Hellemans *et al.*, 1999) while other studies demonstrated an MTs modulation effect on the immune system (Lynes *et al.*, 1993). Associations of MTs and several other diseases (circulatory and sceptic shock, coronary artery disease and Alzheimer's disease) have also been made. In general, an overexpression of MTs indicates a poor prognosis in human health (Hellemans *et al.*, 1999; Simpkins, 2000).

From all these studies, it clearly appears that MTs' role in biological system is most likely multifunctional.

An overview of MTs analysis in seabirds is presented in chapter 6.

References

AASETH, J. & NORSETH, T. (1986). Copper. In: Friberg, L., Nordberg, G.F., Vouk, V.B. (Eds). Handbook on the toxicology of metals. Second edition, Elsevier, 233-254.

ANDREWS, G.K., FERNANDO, L.P., MOORE, K.L., DALTON, T.P., SOBIESKI, R.J. (1996). Avian metallothioneins: structure, regulation and evolution. The Journal of Nutrition 126 (4S): 1317S-1323S.

BARRETT, R.T. & KRASNOV, Y.V. (1996). Recent responses to changes in stocks of prey species by seabirds breeding in the southern Barents Sea. ICES Journal of Marine Science 53: 713-722.

BAYNES, R.D. (1996). Assessment of iron status. Clinical Biochemistry 29 (3): 209-215.

BEDART, J. (1969). Adaptative radiation in Alcidae. Ibis 111: 189-198.

BEDART, J. (1985). Evolution and characteristics of the Atlantic Alcidae. In: Nettleship, D.N. & Birkhead, T.R. (Eds). The Atlantic Alcidae: the evolution, distribution and biology of Auks inhabiting the Atlantic Ocean and adjacent waters areas. Academic Press, London, 1-50.

BEISEL, W.R., PEKAREK, R.S., WANNEMACHER, R.W.JR. (1976). Homeostatic mechanisms affecting plasma zinc levels in acute stress. In: Prasad, A.S. & Oberleas, D. (Eds). Trace elements in human health and disease. Volume II. Essential and toxic elements. Academic Press, 87-106.

BELOPOL’SKII L.O. (1957). Ecology of sea colony birds of the Barents Sea. English translation by Israel Program for Scientific Translations, Jerusalem, 346 pp (translated from Russian 1961).

BEYERSMANN, D. & HECHTENBERG, S. (1997). Cadmium, gene regulation, and cellular signaling in mammalian cells. Toxicology and Applied Pharmacology 144: 247-261.

BIRKHEAD, T.R. (1974). Movement and mortality rates of British Guillemots. Bird Study 21 : 241-254.

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. & TAYLOR, A.M. (1977). Moult of the Guillemot *Uria aalge*. Ibis 119 : 80-85.

BLAKE, B.F. (1984). Diet and fish stock availability as possible factors in the mass death of auks in the North Sea. Journal of Experimental Marine Biology and Ecology 76: 89-103.

BLAKE, B.F., DIXON, T.J., JONES, P.H., TASKER, M.L. (1985). Seasonal changes in the feeding ecology of Guillemots (*Uria aalge*) off North and East Scotland. Estuarine, Coastal and Shelf Science 20: 559-568.

BODKIN, J.L. & JAMESON, R.J. (1991). Patterns of seabird and marine mammal carcass deposition along the central California coast, 1980-1986. Canadian Journal of Zoology 69: 1149-1155.

BORCH-IOHNSEN, B., HOLM, H., JØRGENSEN, A., NORHEIM, G. (1991). Seasonal siderosis in female eider nesting in Svalbard. Journal of Comparative Pathology 104: 7-15.

BOYLE, E.A. (1979). Copper in natural waters. In: Nriagu, J.O. (Ed). Copper in the environment. Part I: ecological cycling. Wiley-Interscience Series, 77-88.

BRAEDSTREET, M.S.W. & BROWN, R.G.B. (1985). Feeding ecology of the Atlantic Alcidae. In: Nettleship, D.N. & Birkhead, T.R. (Eds). The Atlantic Alcidae: the evolution, distribution and biology of Auks inhabiting the Atlantic Ocean and adjacent waters areas. Academic Press, London, 263-318.

BREMMER, I. (1979). Mammalian absorption, transport and excretion of cadmium. In: Webb, M. (Ed). The chemistry, biochemistry and biology of cadmium. Elsevier/North-Holland Biomedical Press, 175-193.

BREMMER, I. (1991). Nutrional and physiologic significance of metallothionein. Methods in Enzymology 205: 25-35.

BRIGGS, K.T., YOSHIDA, S.H., GERSHWIN, M.E. (1996). The influence of petrochemicals and stress on the immune system of seabirds. Regulatory Toxicology and Pharmacology 23: 145-155.

BROWN, R.G.B. (1985). The Atlantic Alcidae at sea. In: Nettleship, D.N. & Birkhead, T.R. (Eds). The Atlantic Alcidae: the evolution, distribution and biology of Auks inhabiting the Atlantic Ocean and adjacent waters areas. Academic Press, London, 383-426.

BURGER, A.E. & PIATT, J.F. (1990). Flexible time-budgets in breeding common murres: buffers against variable prey abundance. Studies in Avian Biology 14: 71-83.

BURGER, A.E. & FRY, M.D. (1992). Effect of oil pollution on seabirds in the northeast Pacific. In: Vermeer, K., Briggs, K.T., Morgan, K.H., Siegel-Causey, D. (Eds). The status, ecology, and conservation of marine birds of the North Pacific. Special publication Canadian Wildlife Service, 254-263.

BURTON, J.D. & STATHAM, P.J. (1990). Trace metals in seawater. In: Furness, R.W. & Rainbow, P.S. (Eds). Heavy metals in the marine environment. CRC Press, 5-25.

CAIRNS, D.K., MONTEVECCHI, W.A., BIRT-FRIESEN, V..L., MACKO, S.A. (1990). Energy expenditures, activity budgets, and prey harvest of breeding common murres. Studies in Avian Biology 14: 84-92.

CALLAWAY, J.C., DELAUNE, R.D., PATRICK, W.H.Jr. (1998). Heavy metals chronologies in selected coastal wetlands from Northern Europe. Marine Pollution Bulletin 36 (1): 82-96.

CAMERON, P. & VON WESTERNHAGEN, H. (1997). Malformation rates in embryos of North Sea fishes in 1991 and 1992. Marine Pollution Bulletin 34 (2): 129-134.

CAMPHUYSEN, C.J. (1989). Beached bird surveys in The Netherlands, 1915-1988. Seabird mortality in the southern North Sea since the early days of oil pollution. (Techn. Rapport Vogelbescherming 1) Werkgroep Noordzee, Amsterdam, 322pp.

CAMPHUYSEN, C.J. (1990a). Massastranding van alken *Alca torda* op de Nederlandse kust, jan-mrt 1990: aantal, leeftijd en oliebesmeuring. Sula 4: 135-138.

CAMPHUYSEN, C.J. (1990b). Massastranding van Alk *Alca torda* en zeekoet *Uria aalge* op de Nederlandse kust, jan-feb 1990. Sula 4: 23-25.

CAMPHUYSEN, C.J. (1995). Leeftijdsbepaling van zeekoet *Uria aalge* en alk *Alca torda* in de hand. Sula 9 (1): 1-22.

CAMPHUYSEN, C.J. (1998). Diurnal activity patterns and nocturanl group formation of wintering common murres in the Central North Sea. Colonial Waterbirds 21 (3): 406-413.

CAMPHUYSEN, C.J. & VAN FRANEKER J.A. (1992). The value of beached bird surveys in monitoring marine oil pollution. Techn. Rapp. Vogelbescherming 10. Vogelbescherming Nederland, Zeist, 191 pp.

CAMPHUYSEN, C.J. & LEOPOLD, M.F. (1994). Atlas of seabirds in the southern North Sea. IBN Research Report 94/6, NIOZ Report 1994-8, Institute for Forestry and Nature Research, Dutch Seabird Group and Netherlands Institute for Sea Research, Texel, 126 pp.

CAMPHUYSEN, C.J. & WEBB, A. (1999). Multi-species feeding associations in North Sea seabirds: jointly exploting a patchy environment. Ardea 87 (2): 177-198.

CAMPHUYSEN, C.J., BARREVELD, H., DAHLMANN, G., VAN FRANEKER, J.A. (1998). Seabirds in the North Sea demobilized and killed by polyisobutylene $(C_4H_8)_n$ (PIB). Marine Pollution Bulletin 38 (12): 1171-1176.

CAMPHUYSEN, C.J., WRIGHT, P.J., LEOPOLD, M., HÜPPOP, O., REID, J.B. (1999). A review of the causes, and the consequences at the population level, of mass mortalities of seabirds. ICES Cooperative Research Report 232: 51-63.

CANESI, L., VIARENGO, A., LEONZIO, C., FILIPPELLI, M., GALLO, G. (1999). Heavy metals and glutathione metabolism in mussel tissues. Aquatic Toxicology 46: 67-76.

CARBONERAS, C. (1988). The auks in the western Mediterranean. Ringing and Migration 9: 18-26.

CARTER, I.C., WILLIAMS, J.M., WEBB, A., TASKER, M.L. (1993). Seabird concentrations in the North Sea: An atlas of vulnerability to surface pollutants. Joint Nature Conservation Committee, Aberdeen, 1-37.

CORK, S.C (2000). Iron storage diseases in birds. Avian Pathology 29: 7-12.

CORK, S.C., ALLEY, M.R., STOCKDALE, P.H.G. (1994). A quantitative assessment of haemosiderosis in wild and captive birds using image analysis. Avian Pathology 24: 239-254.

COUSINS, R.J. (1985). Absorption, transport and hepatic metabolism of copper and zinc: special reference to metallothioneins and ceruloplasmin. Physiological Reviews 65 (2): 238-308.

CRAMP, S. (1985). Handbook of the birds of Europe the Middle East and North Africa. The birds of the Western palearctic. Volume IV. Terns to woodpeckers. Oxford University Press, 168-190.

CROLL, D.A. & MCLAREN, E. (1993). Diving metabolism and thermoregulation in common and thick-billed murres. Journal of Comparative Physiology B 163: 160-166.

DAHLMANN, G., TIMM, D., AVERBECK, C., CAMPHUYSEN, C., SKOV, H., DURINCK, J. (1994). Oiled seabirds – comparative investigations on oiled seabirds and oiled beaches in The Netherlands, Denmark and Germany (1990-93). Marine Pollution Bulletin 28 (5): 305-310.

DAOUST, P.-Y., CONBOY, G., MCBURNEY, S., BURGESS, N. (1998). Interactive mortality factors in common loons from Maritime Canada. Journal of Wildlife Diseases 34 (3): 524-531.

DAS, K., DEBACKER, V., PILLET, S., BOUQUEGNEAU, J-M. (1999). Heavy metals in marine mammals. In: Toxicology of marine mammals. Taylor & Francis Publ. Washington D.C., *in press*.

DEGANGE, A.R., DAY, R.H., TAKEKAWA, J.E., MENDENHALL, V.M. (1993). Losses of seabirds in gill nets in the North Pacific. In : Vermeer, K, Briggs, K.T., Morgan, K.H., Siegel-Causey, D. (Eds). The status, ecology, and conservation of marine birds of the North Pacific. Canadian Wildlife Service Special Publication, Ottawa, 204-211.

DE GROOT, S.J. (1996). The physical impact of marine aggregate extraction in the North Sea. ICES Journal of Marine Science 53:1051-1053.

DETHLESFEN, V., SOFFKER, K., BUTHER, H., DAMM, U. (1996). Organochlorine compounds in marine organisms from the international North Sea incineration area. Archives of Fisheries and Marine Resources 43 (3):215-242.

DIAMOND, G.L. & ZALUPS, R.K. (1998). Understanding renal toxicity of heavy metals. Toxicologic Pathology 26 (1): 92-103.

DIERENFELD, E.S., PINIS, M.T., SHEPPARD, C.D. (1994). Hemosiderosis and dietary iron in birds. Journal of Nutrition 124: 2685S-2686S.

DORRESTEIN, G.M. & VAN DER HAGE, M. (1997). Marine bird necropsy findings. In: Jauniaux, T., Bouquegneau, J.-M., Coignoul, F. (Eds). Marine mammals, seabirds and pollution of marine systems. Presses de la Faculté Vétérinaire de l'Université de Liège, 151-165.

DUCROTOY, J-P. (1999). Indications of change in the marine flora of the North Sea in the 1990s. Marine Pollution Bulletin 38 (8): 646-654.

DUNNET, G.M., FURNESS, R.W., TASKER, M.L., BECKER, P.H. (1990). Seabirds ecology in the North Sea. Netherlands Journal of Sea Research 26 (2-4):387-425.

EISLER, R. (1998). Copper hazards to fish, wildlife, and invertebrates: a synoptic review. Biological Science Report USGS/BSR—1997—0002, 99 pp.

ENCARTA (1996). Atlas Mondial. Microsoft®

ELINDER, C.-G. (1986). Zinc. In: Friberg, L., Nordberg, G.F., Vouk, V.B. (Eds). Handbook on the toxicology of metals. Second edition, Elsevier, 664-679.

ERIKSTAD, K.J. & VADER, W. (1989). Capelin selection by Common and Brunnich's guillemots during the prelaying season. Ornis Scandinavia 20 (2): 151-154.

EVANS, P.G.H. (1982). Associations between seabirds and cetaceans - a review. Mammal Review 12: 187-206.

EVANS, P.G.H. (1990). European cetaceans and seabirds in an oceanographic context. Lutra 33 (2): 95-125.

FABISIAK, J.P., TYURIN, V.A., TYURINA, Y.Y., BORISENKO, G.G., KOROTAEVA, A., PITT, B.R., LAZO, J.S., KAGAN, V. (1999). Redox regulation of copper-metallothionein. Archives of Biochemistry and Biophysics 363 (1): 171-181.

FICHET, D., RADENAC, G., MIRAMAND, P. (1998). Experimental studies of impacts of harbour sediment resuspension to marine invertebrates larvae: bioavailabillity of Cd, Cu, Pb and Zn and toxicity. Marine Pollution Bulletin 36 (7-12): 509-518.

FORRESTER, D.J., DAVIDSON, W.R., LANGE, R.E.Jr., STROUD, R.K., ALEXANDER, L.L., FRANSON, J.C., HASELTINE, S.D., LITTELL, R.C., NESBITT, S.A. (1997). Winter mortality of common loons in Florida coastal waters. Journal of Wildlife Diseases 33:833-847.

FÖRSTNER, U. (1980). Cadmium. In: The Hand book of Environmental Chemistry. 3 A: 59-107.

FOULKES, E.C. (2000). Transport of toxic metals across cell membranes. Proceedings of the Society for Experimental Biology and Medecine 223 (3): 234-240.

FREETHY, R. (1987). Auks: an ornithologist's guide. Facts on File Publication, Oxford, England, 74-104.

FRIBERG, L., KJELLSTRÖM, T., NORDBERG, G.F. (1986). Cadmium. In: Friberg, L., Nordberg, G.F., Vouk, V.B. (Eds). Handbook on the toxicology of metals. Second edition, Elsevier, 130-184.

FRID, C.L.J. & HALL, L.J. (1999). Inferring changes in North Sea benthos from fish stomach analysis. Marine Ecology Progress Series 184 : 183-188.

FRY, M.D. & LOWENSTINE, L.J. (1985). Pathology of common murres and Cassin's auklets exposed to oil. Archives of Environmental Contamination and Toxicology 14: 725-737.

FURNESS, R.W. (1984). Seabird biomass and food consumption in the North Sea. Marine Pollution Bulletin 15 (7) : 244-248.

FURNESS, R.W. (1985). Plastic particles pollution : accumulation by Procellariiform seabirds at Scottish colonies. Marine Pollution Bulletin 16 : 103-106.

FURNESS, R.W. (1989). Declining seabird populations. Journal of Zoology, London 219: 177-180.

FURNESS, R.W. (1993). Birds as monitors of pollutants. In: Furness, R.W. & Greenwood, J.J.D. (Eds). Birds as monitors of environmental changes. Chapman & Hall, 86-143.

FURNESS, R.W. (1996). Cadmium in birds. In: Beyer, W.N., Heinz, G.H., Redmon-Norwood, A.W. (Eds). Environmental contaminants in wildlife: interpreting tissue concentrations. CRC Press, 389-404.

FURNESS, R.W. & HUTTON, M. (1979). Pollutant levels in the great skua *Catharacta skua*. Environmental Pollution 19: 261-268.

FURNESS, R.W. & BIRKHEAD, T.R. (1984). Seabird colony distributions suggest competition for food supplies during the breeding season. Nature 311: 655-656.

FURNESS, R.W. & BARRETT, R.T. (1985). The food requirements and ecological relationships of a seabird community in North Norway. Ornis Scandinavia 16: 305-313.

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. & TASKER, M.L. (1997). Seabird consumption in Sand Lance MSVPA models for the North Sea, and the impact of industrial fishing on seabirds population dynamics. In: Forage fishes in marine ecosystems. Proceeding of the International

Symposium on the role of forage fishes in marine ecosystems. Alaska Sea Grant College Program Report N° 97-01: 147-169. University of Alaska, Fairbanks.

GABRIELSEN, G.W. (1996). Energy expenditure of breeding common murres. In: Montevecchi, W.A. (Ed). Studies of high latitude seabirds. 4. Trophic relationships and energetics of endotherms in cold ocean systems. Occasional Paper of the Canadian Wildlife Service 91: 49-58.

GABRIELSEN, G.W., MEHLUM, F., KARLSEN, H.E. (1988). Thermoregulation in four species of arctic seabirds. Journal of Comparative Physiology B 157: 703-708.

GOLOVKIN, A.N. (1963). Murres (*Uria* species) and Black-legged kittiwakes (*Rissa tridactyla*) fish consumption during the nesting period in the Barents Sea. Zoologicheskii Zhurnal 42 (3): 408-416. (Translated from Russian, 1983: Doenv Tr-2287, Environment Canada, Ottawa).

HALL, W.S., BUSHONG, S.J., HALL, L.W.Jr., LENKEVICH, M.S., PINKNEY, A.E. (1988). Monitoring dissoved copper concentrations in Chesapeake Bay, U.S.A. Environmental Monitoring and Assessment 11: 33-42.

HALLEY, D.J. & HARRIS, M.P. (1993). Intercolony movement and behaviour of immature Guillemots *Uria aalge*. Ibis 135: 264-270.

HALLEY, D.J., HARRIS, M.P. & WANLESS, S. (1995). Colony attendance pattern and recruitment in immature common murres (*Uria aalge*). The Auk 112 (4): 947-957.

HAMER, D.H. (1986). Metallothionein. Annual Review of Biochemistry 55: 913-951.

HARRIS, M.P. & BIRKHEAD, T.R. (1985). Breeding ecology of the Atlantic Alcidae. In: Nettleship, D.N. & Birkhead, T.R. (Eds). The Atlantic Alcidae: the evolution, distribution and biology of Auks inhabiting the Atlantic Ocean and adjacent waters areas. Academic Press, London, 155-204.

HARRIS, M.P. & BAILEY, R.S. (1992). Mortality rates of puffin *Fratercula arctica* and guillemot *Uria aalge* and fish abundance in the North Sea. Biological Conservation 60: 39-46.

HARRIS, M.P. & WANLESS, S. (1985). Fish fed to young Guillemots, *Uria aalge*, and used in display on the Isle of May, Scotland. Journal of Zoology, London 207: 441-458.

HARRIS, M.P. & WANLESS, S. (1988). The breeding biology of Guillemots *Uria aalge* on the Isle of May over a six year period. Ibis 130: 172-192.

HARRIS, M.P. & WANLESS, S. (1990). Moult and colony attendance of auks. British Birds 83: 55-67.

HARRIS, M.P. & WANLESS, S. (1996). Differential responses of Guillemot *Uria aalge* and Shag *Phalacrocorax aristotelis* to a late winter wreck. Bird Study 43:220-230.

HARRIS, M.P., WANLESS, S., BARTON, T.R. (1996). Site use and fidelity in the Common Guillemot *Uria aalge*. Ibis 138: 399-404.

HASCHEK, W.M. & ROUSSEAUX, C.G. (1998). The kidney. In: Haschek, W.M. & Rousseaux, C.G. (Eds). Fundamentals of toxicologic pathology. Academic Press, London, 152-191.

HEDGREN, S. & LINNMAN, A. (1979). Growth of guillemot *Uria aalge* chicks in relation to time of hatching. Ornis Scandinavia 10: 29-36.

HELLEMANS, G., SOUMILLION, A., PROOST, P., VAN DAMME, J., POPPEL, H., BAERT, L., DELEY, M. (1999). Metallothioneins in human kidneys and associated tumors. Nephron 83: 331-340.

HIDALGO, J., BORRáS, M., GARVEY, J., ARMARIO, A. (1990). Liver, brain, and heart metallothionein induction by stress. Journal of Neurochemistry 55: 651-654.

HISLOP, J.R.G., HARRIS, M.P., SMITH, J.G.M. (1991). Variation in the calorific value and total energy content of the lesser sand eel (*Ammodytes marinus*) and other fish preyed on by seabirds. Journal of Zoology, London 224: 501-517.

HONDA, K., YAMAMOTO, Y., HIDAKA, H., TATSTUKAWA, R. (1986). Heavy metal accumulation in Adélie penguin, *Pygoscelis adeliae*, and their variations with the reproductive processes. Memoirs of the National Institute of Polar Research, Spec. Issue 40: 443-453.

HOPE JONES, P.; BARRETT, C.F.; MUDGE, G.P., HARRIS, M.P. (1984). Physical condition of auks beached in eastern Britain during the wreck of February 1983. Bird Study 31: 95-98.

HOUWEN, P.J. (1968). Dénombrement des oiseaux échoués sur une portion de la côte belge pendant l'hiver 1967-1968. Aves 5: 170-177.

HUDSON, P.J. (1985). Population parameters for the Atlantic Alcidae. In: Nettleship, D.N. & Birkhead, T.R. (Eds). The Atlantic Alcidae: the evolution, distribution and biology of Auks inhabiting the Atlantic Ocean and adjacent waters areas. Academic Press, London, 233-261.

HUNT, G.L., BARRETT, R.T., JOIRIS, C., MONTEVECCHI, W.A. (1996). Seabird/fish interactions: an introduction. In: Hunt, G.L. & Furness, R.W. (Eds). Seabird/fish interactions, with particular reference to seabirds in the North Sea. ICES Cooperative Research Report 216: 2-5.

HUTTON, M. (1981). Accumulation of heavy metals and selenium in three seabird species from the United Kingdom. Environmental Pollution (A) 26: 129-145.

IARC (1993). Beryllium, cadmium, mercury, and exposure in the glass manufacturing industry. International Agency for Research on Cancer, Lyon, IARC Monographs on the evaluation of carcinogenic risks to humans, Vol. 58.

IVERSEN, J.A. & KROG, J. (1972). Body temperature in active and resting Charadriiforme birds (murre, puffin and auklets) at differrent ambient temperatures. Norwegian Journal of Zoology 20: 145-148.

JACOB, S.T., GHOSHAL, K., SHERIDAN, J.F. (1999). Induction of metallothionein by stress and its molecular mechanisms. Gene Expression 7: 301.310.

JACQUES, T.G. (1995). La pratique belge et le droit de la mer: la protection de l'environement marin. Revue Belge de Droit International 1: 127-146.

JIANG, L-J, MARET, W., VALLEE, B.L. (1998). The glutathione redox couple modulates zinc tranfer from metallothionein to zinc-depleted sorbitol dehydrogenase. Proceedings of the National Academy of Sciences of the United States of America 95: 3483- 3488.

JAUNIAUX T., BROSENS L., DEBACKER, V., BOUQUEGNEAU J.M., COIGNOUL, F. (1993). Pathology of oiled seabirds at the Belgian coast. Scientific Symposium on the 1993 North Sea Quality Status Report.

JAUNIAUX T., BROSENS L., FARNIR F., MANTECA C., LOSSON B., TAVERNIER J., VINDEVOGEL H., COIGNOUL F. (1996). Mortalité des oiseaux marins lors de l'hiver 1992-1993 le long du littoral belge. Annales de Médecine Vétérinaire 140: 149-159.

JAUNIAUX T., BROSENS L., MEIRE, P., OFFRINGA, H., COIGNOUL, F. (1998). Pathological investigations on guillemots (*Uria aalge*) stranded on the Belgian coast during the winter 1993-94. The Veterinary Record 143: 387-390.

JOIRIS, C. (1972). Observations ornithologiques réalisées dans le sud-est de la Mer du Nord, entre juin 1971 et janvier 1972. 1. Oiseaux marins. Aves 9: 85-103.

JOIRIS, C. (1978). Seabirds recorded in the Northern North Sea in July: the ecological implications of their distribution. Le Gerfaut 68: 419-440.

JOIRIS, C. (1983a). Winter distribution of seabirds in the North Sea: an oceanological interpretation. Le Gerfaut 73: 107-123.

JOIRIS, C. (1983b). Seabirds recorded in the Fladenground area, Northern North Sea, in April, May and June 1976. In: Sündermann & Lenz (Eds). North Sea dynamics. Springer-Verlag, Heidelberg, 676-686.

JØRGENSEN, L.A. & PEDERSEN, B. (1994). Trace metals in fish used for time trend analysis and as environmental indicators. Marine Pollution Bulletin 28 (1): 24-32.

KÄGI, J.H.R. (1991). Overview of metallothionein. Methods in Enzymology 205: 613-626.

KÄGI, J.H.R. (1993). Evolution, structure, and chemical activity of class I metallothioneins: an overview. In: Suzuki, K.T., Imura, N., Kimura, M. (Eds). Metallothionein III: biological role and medical implications. Birkhäuser Verlag, Basel, 29-55.

KHAN, R.A. & RYAN, P. (1991). Long term effects of crude oil on common murres (*Uria aalge*) following rehabilitation. Bulletin of Environmental Contamination and Toxicology. 46: 216-222.

KHAN, R.A. & NAG, K. (1993). Estimation of hemosiderosis in seabirds and fish exposed to petroleum. Bulletin of Environmental Contamination and Toxicology 50: 125-131.

KIM, E.Y., GOTO, R., TANABE, S., TANAKA, H., TATSUKAWA, R. (1998). Distribution of 14 elements in tissues and organs of oceanic seabirds. Archives of Environmental Contamination and Toxicology 35: 638-645.

KLAASSEN, C.D. & LIU, J. (1998). Metallothionein transgenic and knock-out mouse models in the study of cadmium toxicity. The Journal of Toxicological Sciences 23 (Supplement II):97-102.

KLAASSEN, C.D., LIU, J., CHOUDHURI, S. (1999a). Metallothionein: an intracellular protein to protect against Cd toxicity. Annu. Rev. Pharmacol. Toxicol. 39: 267-294.

KLAASSEN, C.D., LIU, J., LIU, Y., HABEEBU, S.S. (1999b). Metallothionein-null mice are susceptible to chronic $CdCl_2$-induced nephropathy: Cd-induced renal injury is not necessarily mediated by CdMT. In: Klaassen, C.D. (Ed). Metallothionein IV. Birkhaüser Verlag, 453-457.

KONDO, Y., RUSNAK, J.M., HOYT, D.G., SETTINERI, C., PITT, B.R., LAZO, J.S. (1997). Enhanced apoptosis in metallothionein null cells. Molecular Pharmacology 52: 195-201.

KUYKEN, E. (1978). Beached birds surveys in Belgium. Ibis 120: 122-123.

LAANE, R.W.P.M. (1992). Background concentrations of natural compounds in rivers, sea water, atmosphere and mussels. Summary of the group reports written during the International Workshop On Background Concentrations of Natural compounds held in The Hague, 6 - 10 April 1992.

LAANE, R.W.P.M., SONNEVELDT, H.L.A., VAN DER WEYDEN, A.J., LOCH, J.P.G., GROENEVELD, G. (1999). Trends in the spatial and temporal distribution of metals (Cd, Cu, Zn and Pb) and organic compounds (PCBs and PAHs) in Dutch coastal zone sediments from 1981 to 1996: a model case study for Cd and PCBs. Journal of Sea Research 41: 1-17.

LANSDOWN, A.B.G. (1995). Physiological and toxicological changes in the skin resulting from the action and interaction of metal ions. Critical Review in Toxicology 25(5): 397-462.

LAUWERYS, R. (1990). Toxicologie industrielle et intoxications professionnelles. Troisième édition, Mason, Paris, 693 pp.

LE GRAND ATLAS, (1993). DeBoeck & Wesmael Eds.

LEIGHTON, F.A. (1993). The toxicity of petroleum oils to birds. Environmental Review 1: 92-103.

LEIGHTON, F.A., PEAKALL, D.B., BUTLER, R.G. (1983). Heinz-body hemolytic anemia from the ingestion of crude oil: primary toxic effect in marine birds. Science 20: 871-873.

LEOPOLD, M.F., WOLF, P.A., HÜPPOP, O. (1992). Food of young and colony-attendance of adult guillemots *Uria aalge* on Helgoland. Helgoländer Meeresunters 46 : 237-249.

LINDEBOOM, H.J. & DE GROOT, S.J. (1999). The effects of the different types of fisheries on the North Sea and Irish Sea benthic ecosystems. Marine Environmental Management Review of 1998 and Future Trends 6 (13): 87-94.

LLOYD, C., TASKER, M.L., PARTRIDGE, K. (1991). The status of seabirds in Britain and Ireland. T & A.D. Poyser, London, 355 pp.

LOCK, J.W., THOMPSON, D.R., FURNESS, R.W., BARTLE, J.A. (1992). Metal concentrations in seabirds of the New Zealand region. Environmental Pollution 75: 289-300.

LORENTSEN, S.-H. & ANKER-NIELSEN, T. (1999). Diet of common murres wintering in the Northern Skagerrak during 1988-1990: variation with sex, age and season. Waterbirds 22 (1): 80-89.

LUOMA, S.N. (1996). The developing framework of marine ecotoxicology: pollutants as a variable in marine ecosystems ? Journal of Experimental Marine Biology and Ecology 200: 29-55.

LYNES, M.A., BORGHESI, L.A., YOUN, J., OLSON, E.A. (1993). Immunomodulatory activities of extracellular metallothionein I. Metallothionein effects on antibody production. Toxicology 85: 161-177.

MARET, W., LARSEN, K., VALLEE, B.L. (1997). Coordination dynamics of biological zinc "clusters" in metallothioneins and in DNA-binding domain of the transcription factor Gal4. Proceedings of the National Academy of Sciences of the United States of America 94: 2233-2237.

MARET, W. & VALLEE, B.L. (1998).Thiolate ligands in metallothionein confer redox activity on zinc clusters. Proceedings of the National Academy of Sciences of the United States of America 95: 3478-3482.

MARGOSHES, M. & VALLEE, B.L. (1957). A Cadmium protein from equine kidney cortex. Journal of the American Chemical Society 79: 1813-1814.

MASON, A.Z. & JENKINS, K.D. (1995). Metal detoxification in aquatic organisms. In: Tessier, A. & Turner, D.R. (Eds). Metal speciation and bioavailability in aquatic systems. IUPAC series on analytical and physical chemistry of environmental systems, 479-608.

MELVIN, E.F., PARRISH, J.K., CONQUEST, L.L. (1999). Novel tools to reduce seabird bycatch in coastal gillnet fisheries. Conservation Biology 13 (6): 1386-1397.

MONAGHAN, P., WALTON, P., WANLESS, S., UTTLEY, J.D., BURNS, M.D. (1994). Efffects of prey abundance on the foraging behaviour, diving efficiency and time allocation of breeding Guillemots *Uria aalge*. Ibis 136: 214-222.

MONAGHAN, P., WRIGHT, P.J., BAILEY, M.C., UTTLEY, J.D., WALTON, P., BURNS, M.D. (1996). The influence of changes in food abundance on diving and surface-feeding seabirds. In: Montevecchi, W.A. (Ed). Studies of high latitude seabirds. 4. Trophic relationships and energetics of endotherms in cold ocean systems. Canadian Wildlife Service, Occasional Paper 91: 10-19.

MONTEVECCHI, W.A. & PIATT, J. (1984). Composition and energy content of mature inshore spawning capelin (*Mallotus villosus*): implications for seabird predators. Comparative Biochemistry and Physiology 78A (1):15-20.

MOUM, T., JOHANSEN, S., ERIKSTAD, K.E., PIATT, J.F. (1994). Phylogeny and evolution of the auks (subfamily Alcinae) based on mitochondrial DNA sequences. Proceedings of the National Academy of Sciences of the United States of America 91: 7912-7916.

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.

MURRAY, S., WANLESS, S., HARRIS, M.P. (1994). The effects of fixed salmon *Salmo salar* nets on guillemot *Uria aalge* and razorbill *Alca torda* in northeast Scotland in 1992. Biological Conservation 70: 251-256.

NETTLESHIP, D.N. & EVANS P.G.H. (1985) Distribution and status of the Alcidae. In: Nettleship D.N. & Birkhead, T.R. (Eds). The Atlantic Alcidae: the evolution, distribution and biology of the Auks inhabiting the Atlantic Ocean and adjacent water areas. Academic Press, London, 84-88.

NICHOLSON, J.K. & OSBORN, D. (1983). Kidney lesions in pelagic seabirds with high tissue levels of cadmium and mercury. Journal of Zoology, London 200: 99-118.

NICHOLSON, J.K., KENDALL, M.D., OSBORN, D. (1983). Cadmium and mercury nephrotoxicity. Nature 304: 633-635.

NISBET, I.C.T. (1994). Effect of pollution on marine birds. Birdlife Conservation Series 1: 8-25.

NRIAGU, J.O. (1979). Copper in the atmosphere. In: Nriagu, J.O. (Ed). Copper in the environment. Part I: ecological cycling. Wiley-Interscience Series, 43-75.

NORHEIM, G. & BORCH-IOHNSEN, B. (1990). Chemical and morphological studies of liver from eider (*Somateria mollissima*) in Svalbard with special reference to the distribution of copper. Journal of Comparative Pathology 102: 457-466.

NORTH SEA TASK FORCE REPORT (1993a). North Sea Subregion 4: assessment report (Netherlands, Belgium, France, Germany and United Kingdom). Oslo and Paris Commission (Olsen & Olsen, Eds), 195 pp.

NORTH SEA TASK FORCE REPORT (1993b). North Sea Subregion 3b: assessment report: United Kingdom. Oslo and Paris Commission (Olsen & Olsen, Eds), 154 pp.

OBERLEAS, D. & PRASAD, A.S. (1976). Factors affecting zinc homeostasis. In: Prasad, A.S. & Oberleas, D. (Eds). Trace elements in human health and disease: Volume II. Essential and toxic elements. Academic Press, 155-161.

OFFRINGA, H., SEYS, J., VAN DEN BOSSCHE, W., MEIRE, P. (1996). Seabirds on the Channel doormat. Le Gerfaut 86: 3-71.

OKA, N. & OKUYAMA, M. (2000). Nutritional status of dead oiled auklets (*Cerorhinca monocerata*) in the Southern Japan Sea. Marine Pollution Bulletin 40 (4): 340-347.

OSBORN, D. (1979). Seasonal changes in the fat protein and metal content of the liver of the starling *Sturnus vulgaris*. Environmental Pollution 19: 145-155.

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 64C: 61-67.

PAIS, I. & BENTON JONES, J. Jr. (1997). The handbook of trace elements. Boca Raton, Florida, 223 pp.

PEAKALL, D.B., JEFFREY, D.A., MILLER, D.S. (1985). Weight loss of herring gulls exposed to oil and oil emulsion. Ambio 14 (2): 108-110.

PEDERSEN, B. (1996). Metal concentration in biota in the North Sea changes and causes. ICES Journal of Marine Sciences 53: 1008-1013.

PETERSON, P.J. & ALLOWAY, B.J. (1979). Cadmium in soils and vegetation. In: Webb, M. (Ed). The chemistry, biochemistry and biology of cadmium. Elsevier/North-Holland Biomedical Press, 45-92.

PHILIPPART, C.J.M. (1998). Long-term impact of bottom fisheries on several by-catch species of demersal fish and benthic invertebrates in the south-eastern North Sea. ICES Journal of Marine Science 55: 342-352.

PIATT, J.F. (1990). The aggregative response of common murres and Atlantic puffins to schools of capelin. Studies in Avian Biology 14: 36-51.

PIATT, J.F. & NETTLESHIP, D.N. (1985). Diving depths of four Alcids. The Auk 102: 293-297.

PIATT, J.F. & NETTLESHIP, D.N. (1987). Incidental catch of marine birds and mammals in fishing nets off Newfoundland, Canada. Marine Pollution Bulletin 18 (6B): 344-349.

PIATT, J.F. & FORD, R.G. (1996). How many seabirds were killed by the Exxon Valdez oil spill ? American Fisheries Society Symposium 18: 712-719.

PISCATOR, M. (1976). The chronic toxicity of cadmium. In: Prasad, A.S. & Oberleas, D. (Eds). Trace elements in human health and disease. Volume II. Essential and toxic elements. Academic Press, 431-441.

PLASMAN, C. (1998). The state of the marine environment of the North Sea and of the Baltic Sea: a comparison in relation to dangerous substances. The International Journal of Marine and Coastal Law, 13 (3): 325-336.

RADACH, G. & KEYER, K. (1997). A cadmium budget for the German Bight in the North Sea. Marine Pollution Bulletin 34 (6): 375-381.

RATTNER, B.A. & JEHL, J.R.Jr. (1997). Dramatic fluctuations in liver mass and metal content of Eared grebes (*Podiceps nigricollis*) during autumnal migration. Bulletin of Environmental Contamination and Toxicology 59: 337-343.

REGEHR, H.M. & RODWAY, M.S. (1999). Seabird breeding performance during two years of delayed capelin arrival in the northwest Atlantic: a multi-species comparison. Waterbirds 22 (1): 60-67.

RIORDAN, J.F. & VALLEE, B.L. (1976). Structure and function of zinc metalloenzymes. In: Prasad, A.S. & Oberleas, D. (Eds). Trace elements in human health and disease. Volume II. Essential and toxic elements. Academic Press, 227-256.

ROESIJADI, G. (1992). Metallothioneins in metal regulation and toxicity in aquatic animals. Aquatic Toxicology 22: 81-114.

ROESIJADI, G. (1996). Metallothionein and its role in toxic metal regulation. Comparative Biochem.istry and Physiology 113 C: 117-123.

ROWE, S., JONES, I.L., CHARDINE, J.W., ELLIOT, R.D., VEITCH, B.G. (2000). Recent changes in the winter diet of murres (*Uria spp.*) in coastal Newfoundland waters. Canadian Journal of Zoology 78: 495-500.

SALOMONSEN, F. (1944). The Atlantic Alcidae. The seasonal and geographical variation of the auks inhabiting the Atlantic Ocean and adjacent waters. Göteborgs Kungliga Vetenskaps - och Vitterhets – Samhälles Handlingar, Serie B 3(5): 1-138.

SCHEUHAMMER, A.M. (1987). The chronic toxicity of Aluminium, Cadmium, Mercury, and Lead in birds: a review. Environmental Pollution 46: 263-295.

SCHEUHAMMER, A.M. (1991). Effects of acidification on the availability of toxic metals and calcium to wild birds and mammals. Environmental Pollution 71: 329-375

SEYS, J. & MEIRE, P. (1992). Resultaten stookolieslachtoffer-tellingen langs de Vlaamse kust in de periode Januari-April 1992. Rapport Instituut voor Natuurbehoud A92.084, 18 pp.

SEYS, J., OFFRINGA, H., VAN WAEYENBERG, J., MEIRE, P., KUIJKEN, E. (2000). Characteristics of seabirds on the Channel doormat: analysis of Belgian beached bird surveys. *In preparation.*

SHERIDAN, R. & PALMART, L. (1988). Analyse de l'échouage et des causes de mortalité d'oiseaux marins récoltés sur la côte Belge entre avril 1986 et mars 1987. Aves 25 (3/4): 153-170.

SIMPKINS, C.O. (2000). Metallothioneins in human disease. Cellular and Molecular Biology 46 (2): 465-488.

SKOV, H., DURINCK, J., DANIELSEN, F., BLOCH, D. (1995). Co-occurrence of cetaceans and seabirds in the Northeast Atlantic. Journal of Biogeography 22: 71-88.

SKOV, H., CHRISTENSEN, K.D., DURINCK, J. (1996). Trends in marine oil pollution in Denmark 1984-1995. Arbejdsrapport n° 75, 62 pp.

SPRING LOWELL (1971). A comparison of functional and morphological adaptations in the Common Murre (*Uria aalge*) and Thick-billed Murre (*Uria lomvia*). Condor 73:1-27.

STEWART, F.M. & FURNESS, R.W. (1998). The influence of age on cadmium concentrations in seabirds. Environmental Monitoring and Assessment 50: 159-171.

STEWART, F.M., THOMPSON, D.R., FURNESS, R.W., HARRISSON, N. (1994). Seasonal variation in heavy metal levels in tissues of common guillemot, *Uria aalge* from northwest Scotland. Archives of Environmental Contamination and Toxicology 27: 168-175.

STEWART, F.M., FURNESS, R.W., MONTEIRO, L.R. (1996). Relationships between heavy metal and metallothionein concentrations in Lesser Black-backed Gulls, *Larus fuscus*, and Cory's Shearwater, *Calonectris diomedea*. Archives of Environmental Contamination and Toxicology 30: 299-305.

STEWART, F.M., MONTEIRO, L.R., FURNESS, R.W. (1997). Heavy metal concentrations in Cory's shearwater, *Calonectris diomedea*, fledglings from the Azores, Portugal. Bulletin of Environmental Contamination and Toxicology 58: 115-122.

STEWART, F.M., PHILLIPS, R.A., BARTLE, J.A., CRAIG, J., SHOOTER, D. (1999). Influence of phylogeny, diet, moult schedule and sex on heavy metal concentrations in New Zealand Procellariiformes. Marine Ecology Progress Series 178: 295-305.

STORER, R.W. (1952). A comparison of variation, behaviour and evolution in the seabird genera *Uria* and *Cepphus*. University of California Publications in Zoology 52: 121-222.

STORER, R.W. (1964). Auk. In: Sir Landsborough Thomson, A. (Ed). A new dictionary of birds. Nelson, London, 67-69.

STOWE, T.J. & UNDERWOOD, L.A. (1984). Oil spillages affecting seabirds in the United Kingdom, 1966-1983. Marine Pollution Bulletin 15(4): 147-152.

STURNIOLO, G.C., DI LEO, V., BAROLLO, M., FRIES, W., MAZZON, E., FERRONATO, A., D'INCA, R. (2000). The many functions of zinc in inflammatory conditions of the gastrointestinal tract. Journal of Trace Elements in Experimental Medecine 13 (1): 97-104.

SWENNEN, C. & DUIVEN, P. (1977). Size of food objects of three fish-eating seabird species: *Uria aalge, Alca torda and Fratercula arctica* (Aves, Alcidae). Netherlands Journal of Sea Research 11: 92-98.

SWENNEN, C. & DUIVEN, P. (1991). Diving speed and food-size selection in common guillemots, *Uria aalge*. Netherlands Journal of Sea Research 27 (2): 191-196.

TASKER, M. & BECKER, P.H. (1992). Influences of human activities on seabirds populations in the North Sea. Netherlands Journal of Aquatic Ecology 26 (1): 59-73.

TASKER, M. & FURNESS, R.W. (1996). Estimation of food consumption by seabirds in the North Sea. In: Hunt, G.L. & Furness, R.W. (Eds). Seabird/fish interactions, with particular reference to seabirds in the North Sea. ICES Cooperative Research Report 216: 6-42.

TASKER, M.L., WEBB, A., HALL, A.J., PIENKOWSKI, M.W., LANGSLOW, D.R. (1987). Seabirds in the North Sea. Final Report of Phase 2 of the Nature Conservancy Council Seabird at sea Project, Peterborough, 336 pp.

THOMPSON, D.R. (1990). Metal levels in marine vertebrates. In: Furness, R.W. & Rainbow, P.S. (Eds). Heavy metals in the marine environment. CRC Press, 143-182.

TROMP, D. & WIERIKS, K. (1994). The OSPAR Convention: 25 years of North Sea protection. Marine Pollution Bulletin 29 (6-12): 622-626.

TUCK, L. (1961). The murres: their distribution, populations and biology - a study of the case *Uria*. Queen's printer, Ottawa, Canada.

UTTLEY, J.D., WALTON, P., MONAGHAN P., AUSTIN, G. (1994). The effects of food abundance on breeding performance and adult time budgets of Guillemots *Uria aalge*. Ibis 136: 204-212.

VAN FRANEKER, J.A. (1985). Plastic ingestion in the North Atlantic fulmar. Marine Pollution Bulletin 16 (9): 367-369.

VAN GOMPEL, J. (1989). Vogels in Vlaanderen. Vlaamse Avifauna Commissie, 233-237.

VAN PELT, T.I., PIATT, J.F., LANCE, B.K., ROBY, D.D. (1997). Proximate composition and energy of some North Pacific forage fishes. Comparative Biochemistry and Physiology 118A 4: 1393-1398.

VAROUJEAN , D.H., SANDERS, S.D., GRAYBILL, M.R., SPEAR, L. (1979). Aspects of common murre breeding biology. Pacific Seabird Group Bulletin 6: 28.

VENUGOPAL, B & LUCKEY, T.P. (1975). Toxicology of non-radioactive heavy metals and their salts. In: Coulston, F. & Korte, F. (Eds). Heavy metal toxicity, safety and hormology. Georg Thieme Publishers, Stuggart, Academic Press, New York, 4-73.

VIARENGO, A. & NOTT, J. (1993). Mechanisms of heavy metal cation homeostasis in marine invertebrates. Comparative Biochemistry and Physiology 104C: 355-372.

VIARENGO, A., BLASCO, J., BURLANDO, B., PONZANO, E., MARCHI, B., TRIELLY, F. (1998). Metallothionein and oxidative stress in marine organisms. In: Widdows, J. & Luoma, S.N. (Eds). Pollutant responses in marine organisms (PRIMO 9). Marine Environmental Research, Special Issue 46: 606-607.

WALSH, P.M. (1990). The use of seabirds as monitors of heavy metals in the marine environment. In: Furness, R.W. & Rainbow, P.S. (Eds). Heavy metals in the marine environment. CRC Press: 183-204.

WANLESS, S., MORRIS, J.A., HARRIS, M.P. (1988). Diving behaviour of Guillemot *Uria aalge*, Puffin *Fratercula arctica* and Razorbill *Alca torda* as shown by radio-telemetry. Journal of Zoology, London 216:73-81.

WANLESS, S.., HARRIS, M.P., GREENSTREET, S.P.R. (1998). Summer sand eels consumption by seabirds breeding in the Firth of Forth, south-east Scotland. ICES Journal of Marine Science 55: 1141-1151.

WARD, R.J., IANCU, T.C., HENDERSON, G.M., KIRKWOOD, J.R., PETERS, T.J. (1988). Hepatic iron overload in birds: analytical and morphological studies. Avian Pathology 17: 451-464.

WEBB, M. (1977). Metabolic targets of metal toxicity. In: Brown, S.S. (Ed). Clinical chemistry and chemical toxicity of metals. Elsevier, 51- 64.

WENZEL, C. & GABRIELSEN, G.W. (1995). Trace element accumulation in three seabird species from Hornøya, Norway. Archives of Environmental Contamination and Toxicology 29: 198-206.

WOLFF, W.J. (2000a). The south-eastern North Sea: losses of vertebrate fauna during the past 2000 years. Biological Conservation 95: 209-217.

WOLFF, W.J. (2000b). Causes of extirpations in the Wadden Sea, an estuarine area in The Netherlands. Conservation Biology 14 (3): 876-885.

WRIGHT, P. & TASKER, M.L. (1996). Analysis of fish consumption by seabirds by age class of prey fish. In: Hunt, G.L. & Furness, R.W. (Eds). Seabird/fish interactions, with particular reference to seabirds in the North Sea. ICES Cooperative Research Report 216: 42-44.

WRIGHT, P.J. & BEGG, G.S. (1997). A spatial comparison of common guillemots and sand eels in Scottish waters. ICES Journal of Marine Science 54: 578-592.

YDENBERG, R.C. (1989). Growth-mortality trade-offs and the evolution of juvenile life histories in the Alcidae. Ecology, 70 (5): 1494-1506.

ZDZIARSKI, J.M., MATTIS, M., BUSH, M.R., MONTALI, R.J. (1994). Zinc toxicosis in diving ducks. Journal of Zoological Wildlife Medecine 25 (3): 438-445.

ZOUN, P.E.F., BAARS, A.J., BOSHUIZEN, R.S. (1991). A case of seabirds mortality in The Netherlands during the winter of 1988/1989 caused by spillage of nonylphenol and vegetable oils. CDI-rapport n°H121519. Abstract. NGZ/NSO Workshop, April 1991, Rijswijk.

Chapter 2

Ecotoxicological and pathological studies of common guillemots *Uria aalge*, beached on the Belgian coast during six successive wintering periods (1989-90 to 1994-95).

Based on the manuscript by : V. Debacker[1], L. Holsbeek[2], G. Tapia [2], S. Gobert[1], C. R. Joiris[2], T. Jauniaux[3], F. Coignoul[3] and J.M. Bouquegneau[1].

[1]. Oceanology, University of Liège, B6 Sart Tilman, B-4000, Belgium.

[2]. Laboratory for Ecotoxicology and Polar Ecology, Free University of Brussels (VUB), Pleinlaan 2, B-1050, Brussels, Belgium.

[3]. Pathology Department of the Veterinary College, University of Liège, B43 Sart Tilman, B-4000, Belgium.

Diseases of Aquatic Organisms (1997), 29 : 159-168.

Contents

Ecotoxicological and pathological studies of common guillemots *Uria aalge*, beached on the Belgian coast during six successive wintering periods (1989-90 to 1994-95).

Abstract

During six successive wintering periods, 727 common guillemots *Uria aalge* were recovered from Belgian beaches. One third of the birds were already dead, the rest passed through rehabilitation centres where they eventually died. All birds were monitored for general condition (body mass, fat reserves), eventual status of oiling and pathological changes (cachexia, **a**cute **h**emorrhagic **g**astro-**e**nteropathy - GEAH -), 339 birds were sampled for trace metals (total and organic Hg, Cu, Zn, Fe, Cd) and PCB analysis. Oiling is still a major cause of death for wintering pelagic seabirds: half of the birds showed signs of external or internal oiling, probably a still greater number of oiled birds never reach the shores. Although a low body mass should be a normal winter condition for wintering guillemots, pathology results showed that three quarters of the studied animals were in a state of cachexia with emaciated pectoral muscles and lowered muscle lipid contents. Elevated levels of Cu, Zn, Hg and PCBs were linked to the state of cachexia and may well represent an additional stress factor leading to debilitation and death of part of the wintering guillemot population.

Introduction

Although a relatively small ecosystem, the North Sea is known for its high fish productivity and catches. However, oil refineries, steelworks, metallurgy, chemical and paper industries form a dense network in the adjacent countries with subsequent busy shipping routes. During the last decades, offshore gas and oil industries have developed rapidly.

To assess the human impact on this complex ecosystem, the Belgian authorities, within the frame of the 3rd European North Sea Conference, promoted a programme to monitor the health and causes of death of seabirds and marine mammals. Emphasis was particularly put on seabirds which are found dead or dying on beaches in far larger numbers than are marine mammals. With a population of about ten million wintering birds, the North Sea is one of the world's major areas for sea, shore and water birds (Birkhead, 1974; Mead, 1974; Bourne & Vauk, 1988; Dunnet *et al.*, 1990; North Sea Task Force Report 1993a,b). Pelagic species - petrels, auks and gannets - are particularly sensitive to ecosystem alteration such as depletion of fish stocks, oil spills, breeding sites destruction, and chronic or acute organochlorine and heavy metals pollution (Bourne & Vauk, 1988; Dunnet *et al.*, 1990; Carter *et al.*, 1993). In particular, oiling is known to be a severe threat to wintering seabirds (Mead & Baillie, 1981; Stowe & Underwood, 1984; Camphuysen & van Franeker, 1992; Carter *et al.*, 1993; Dahlmann *et al.*, 1994). The common guillemot *Uria aalge*, outnumbers by far all other wintering species. As a consequence, it became the focus of this study.

Seabird mortality and, in particular, winter strandings have been carefully reported and monitored along the Belgian coast (Kuyken, 1978) and the neighbouring countries (*e.g.* Camphuysen & van Franeker, 1992). Camphuysen & Leopold (1994) estimated the number of wintering guillemots in the 130,000 km^2 southern North Sea area at about 235,000 individuals for the 1984 and 1987 October-November peak period. A decline in density occurs around

February-March as the birds move back towards the breeding grounds. To what extent birds dying at sea contribute to this decline is unclear, as the percentage of these birds finally reaching the shores is unknown.

Seabirds are likely candidates to accumulate toxic pollutants (organochlorines and heavy metals) and have been widely used as bioindicators (Muirhead & Furness, 1988; Ohlendorf & Fleming, 1988; Walsh, 1990; Thompson, 1990; Elliot *et al.*, 1992; Thompson *et al.*, 1992; Stewart *et al.*, 1994; Burger & Gochfeld, 1995; Wenzel & Gabrielsen, 1995). Long term chronic effects of contaminants may have severe consequences on reproduction, disease, stress susceptibility (immuno-suppression) and behaviour patterns (Scheuhammer, 1987; Peakall, 1992). Despite extensive information about heavy metals and organochlorine levels in seabirds, few papers have considered the possible links with pathological findings. The aim of this paper is to combine ecotoxicological data and the most severe pathological ones (cachexia; **a**cute and **h**emorrhagic **g**astro-**e**nteropathy - GEAH -) in order to evaluate the possible causes of death of wintering common Guillemots. Preliminary results concerning the mean heavy metal content of the birds collected between 1990 and 1993 suggested high levels of Cu, Zn and Hg (Bouquegneau *et al.*, 1994).

Material and methods

Collection and storage

A regular and systematic collection of stranded seabirds was organised along the 62.1 kilometers of the Belgian shore during 6 successive winters (1989-90 to 1994-95). Two hundred and fifty one dead guillemots were collected from the beaches, 476 still alive went through rehabilitation centres where they eventually died. Putrified specimens were discarded. Collected carcasses were kept frozen until necropsy was performed at the Pathology Department of the Veterinary College, Liège University, using a consistent protocol (Dorrestein & van der Hage, 1993). They were weighed, oil contamination on plumage and/or in intestinal tract and lesions were noted. Nutritional state, absence of subcutaneous fat and light to severe atrophy of pectoral muscle (visible signs of cachexia) were evaluated on a range from 0 to 3; specifically: 0: presence of subcutaneous fat, normal pectoral muscle; 1: absence of fat and slight pectoral muscle atrophy; 2: moderate pectoral muscle atrophy; 3: severe pectoral muscle atrophy. For statistics, group 0 was tested against groups 1, 2, and 3 to compare normal v/s cachectic birds. Necropsy technique involved opening of bodily cavities, dissection of the digestive tract, examination of the respiratory, urinary and genital systems (Jauniaux *et al.*, 1996). Intestinal serosal surface congestion, hyperaemic and thickened intestinal wall, and hemorrhagic content were used as parameters for **a**cute and **h**emorrhagic **g**astro-**e**nteropathy - GEAH - diagnosis (Dorrestein & van der Hage, 1996). Parasites were identified on 248 guillemots and have been previously reported by Brosens *et al.* 1996. Respiratory tract mycetes (*Aspergillus spp.*) have been identified on 7 guillemots out of 198 (Jauniaux & Coignoul, 1994). For bacteriology, all 727 birds have been evaluated for evidence of intestinal salmonellosis following a classic isolation procedure reported elsewhere (Jauniaux *et al.*, 1996). Three birds were positive for *Salmonella* (two cases of *S. enteritidis* and one case of undetermined *Salmonella spp.*). Histopathology was restricted to lesions observed at necropsy. Most lesions were histolytic and bore freezing artifacts. The only significant lesions seen in guillemots were in relation with infectious agents such as *Aspergillus spp.* (see above). No test was used for virus isolation. Two age classes were considered based on the presence of cloacal *bursa fabricii* (Camphuysen & van Franeker, 1992): class I comprising juvenile (1st winter) and immature (2nd and 3rd winter) birds; class II (4th winter and on) consisting of mature, but not necessarily breeding birds. From a total of 727 birds, 339 (170 beached dead, 169 rehabilitation centres) were dissected and samples of liver, kidney and pectoral muscle were collected for analysis of total Hg, organic Hg, polar lipids and PCBs (Laboratory for Ecotoxicology and Polar Ecology of Brussels Free University) and heavy metals, metallothioneins and total lipid (Oceanology, University of Liège).

Total mercury and organic mercury analyses

Total mercury analyses were performed by specific atomic absorption spectrometry using a Perkin-Elmer MAS-50 Mercury analyser after the method described by Hatch & Ott (1968), modified by Bouquegneau (1973).

Organic mercury (MeHg) concentrations were measured by ECD semi-capillary gas chromatography on a Packard 437 following a toluene three step extraction (Uthe *et al.*, 1972). Fresh weight/dry weight ratio was determined by lyophilising. Mercury concentrations were expressed as µg/g dry weight.

Quality control measurements for both total and organic mercury included replicate analysis resulting in coefficients of variation <10 % and analysis of certified reference material (DORM-1, NRC Canada) with a variation in the measurement up to 10 % at the most. Limits of detection were 0.01 µg and 0.02 ng respectively, corresponding to 0.01 and 0.02 µg/g dw for an average 1 g sample.

Other trace element analysis

Atomic absorption spectrophotometry (ARL 3510) was used to determine heavy metal concentrations (Cu, Zn, Cd, Fe). Pb, Ni, Cr and Ti contents were also determined but the results most often were below the detection limits and will not be discussed. After being weighed and dried during 48 hours at 110°C, samples were digested with a mixed solution of chloric and nitric acids (1:3,v:v) and slowly heated to 100°C until complete digestion. The samples were then diluted, filtered and analysed. Parallel to the samples, a set of certified material samples (CRM 278 Community Bureau of Reference, Commission of the European Communities) was also analysed to ensure the method's sensitivity. Recoveries ranged from 92 to 102 % for Cu, Zn and Fe and 80 % for Cd. Limits of detection were 0.01µg/g dw for Cu, 0.33 for Zn and 0.22 for Cd. Concentrations are expressed as µg/g dry weight.

PCB analysis

PCB residues were determined by ECD-gas chromatography on a Shimadzu GC14A using a 30 m fused silica CPSil 8CB capillary column following an hexane and florisil clean-up. PCBs were identified using a congener mixture including IUPAC congeners 28, 31, 52, 101, 118, 138, 153, 156, 170, 180 and 194. Results were expressed as µg/g dry weight. Since the sample PCB patterns did not sufficiently coincide with Aroclor 1254 or 1260 patterns, results were expressed as ΣPCB, or the sum of the 10 individually identified congeners, which represent ± 35% of the total PCB load.

Sample preparation and lipids analysis

The method used for the total lipids extraction was described by Barnes & Blackstock (1973). The polar lipid content was determined gravimetrically after lipid hexane extraction included in the PCB procedure. Total and polar lipids are expressed as g/g dw.

Statistical analysis

All statistical tests were performed using Statistica® for Windows 5.1 computer programme. Tissue concentrations for each metal were tested to fit a normal distribution using Kolmogorov-Smirnov one-sample tests. In case of normal distribution, data were analysed using a t-test. When data significantly differed from a normal distribution, a non parametric test (Mann-Whitney U-test) was used. Differences were considered significant when $p<0.01$.

Results and discussion

None of the birds recovered in the present study were ringed, so that no information was available on their origin and/or their wandering prior to death. This situation most probably reflects the fact that only a small proportion of birds are ringed and that not all dying birds are washed ashore (Pionneau, 1987; Camphuysen & van Franeker, 1992). Nevertheless, a small number of ringed guillemots (n = 27) have been found in Belgium during the 1980s and 1990s and were mainly of Scottish origin (17/27); only a minor fraction came from Germany, Sweden, The Netherlands, the South of England and Ireland (W. Roggeman, *pers. com.*, appendix table 3). Recoveries of guillemots during the 1980s in The Netherlands revealed that a majority of birds had been ringed in Scotland (Camphuysen & van Franeker, 1992). With the necessary caution based on the fact that ringing efforts are not the same in all countries, it still seems reasonable to assume that most of the guillemots collected during the past six years originated from the Scottish area. Several studies show that guillemots have no clear migration pattern, but rather disperse at sea, and that immature individuals are likely to show a higher mortality rate than adults birds (Birkhead, 1974; Mead, 1974; Nettleship & Evans, 1985; Lloyd *et al.*, 1991). Both Landsborough (1953) and Mead (1974) showed that guillemots ringed at colonies on the eastern coasts of England and Scotland had moved through the English Channel and the southern part of the North Sea. Aerial and ship surveys in the southern North Sea, clearly indicate that large numbers of guillemots enter this area by October-November and move out again by February-March (Camphuysen & Leopold, 1994).

A sample of 339 guillemots was fully investigated. During the six winters included, 89 % of the birds were collected from January to March (figure 1). Peak densities (number of guillemots per km^2 sea surface) in the southern North Sea were recorded from October to January (Camphuysen & Leopold, 1994). High densities, probably combined with severe environmental constraints such as low temperatures, storms and starvation, provoke an important mortality during the second half of the wintering period. A large proportion of the birds were oiled, either externally or both externally and internally, or showed clear signs of exhaustion, with emaciated pectoral muscle and very little or absence of abdominal and subcutaneous fat, two distinctive features of cachexia, a long and chronic condition (table 1).

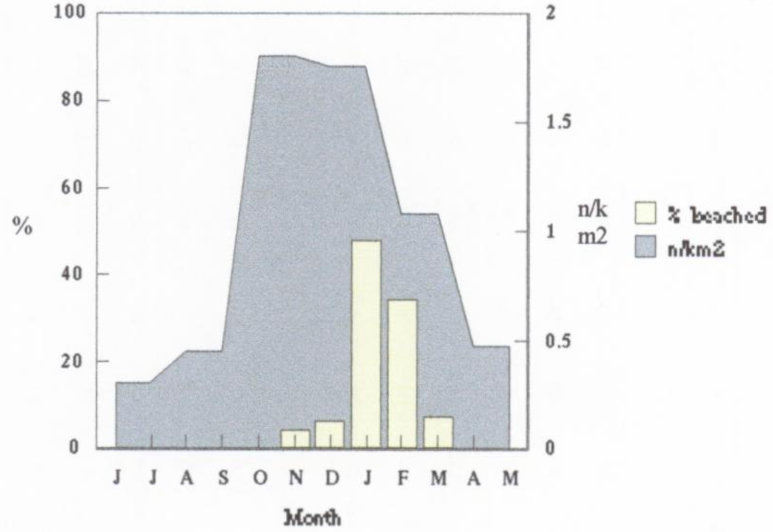


Figure 1: overall stranding (percentage of total number) of guillemots (this work) compared to their densities (number/km²) in the southern North Sea (Camphuysen & Leopold, 1994).

Significant differences appeared for Zn, Fe, total Hg, organic Hg and PCBs between dead birds from the beach and those provided by rehabilitation centres (table 2). These high levels of pollutants in rehabilitated birds are not likely to result from a decrease of body mass but probably from dietary changes. This indeed is confirmed by the fact that $\delta^{13}C$ content of the tissues were lower in rehabilitated guillemots (Caulle *et al.*, unpublished results). For this reason, from the third winter on, we decided to focus on individuals found dead only, considering that birds that passed through rehabilitation centres could be an important bias. The following discussion therefore only refers to animals washed ashore dead. Nevertheless, this sample is not necessarily fully representative for the 'natural' population.

Most of the birds were oiled (55 %) and cachectic (76 %) (table 1). Sixty-one percent had developed acute hemorrhagic gastro-enteropathy. Thirty-one percent were oiled externally and internally, 24 % showed only external traces; 45 % showed no signs of oiling. Oiling is known to be a major cause of death for wintering guillemots entering the fairly polluted southern North Sea (Stowe & Underwood, 1984; Camphuysen & Leopold, 1994; Dahlmann *et al.*, 1994; Camphuysen, 1995). Partial or extensive oiling necessarily leads to starvation, debilitation and subsequent death, and eventual stranding.

		Age		Sex		Cachexia		Gastro-enteritis			Oiling	
		Class I	Class II									
	n	Juv. + imm.	Adult	Male	Female	-	+	-	+	no	E.	E + I
Beached												
Winter 1989-1990	48	77	23	62	38	23	77	54	46	0	11	89
Winter 1990-1991	31	45	55	60	40	18	82	54	46	9	0	91
Winter 1991-1992	12	67	33	42	58	30	70	60	40	33	0	67
Winter 1992-1993	75	54	46	49	51	12	88	34	66	56	24	20
Winter 1993-1994	74	70	30	57	43	30	70	36	64	63	31	6
Winter 1994-1995	11	70	30	80	20	27	73	9	91	36	67	0
All	251	65	35	56	44	24	76	39	61	45	24	31
Centre												
Winter 1989-1990	83	67	33	69	31	13	87	58	42	0	31	69
Winter 1990-1991	122	63	38	50	50	40	60	52	48	0	12	88
Winter 1991-1992	64	83	17	65	35	37	63	60	40	12	23	65
Winter 1992-1993	116	81	19	50	50	9	91	43	57	34	20	46
Winter 1993-1994	76	67	33	63	37	18	82	43	57	49	42	9
Winter 1994-1995	15	60	40	64	36	40	60	47	53	53	47	0
All	476	75	25	61	39	26	74	55	45	13	23	64

Table 1: percentages of class I (juvenile and immature) and class II (adult), male and female, non cachectic (-) and cachectic (+), acute hemorrhagic gastro-enteropathy negative (-) and positive (+), oiling : no oiling, external only (E.) and external and internal oiling (E.+I.), of guillemots, either collected directly from the beach (Beached) or after a stay in a rehabilitation centre (Centre).

	Beach	Centre	p =
Body mass	725 ± 125 700 440-1180 *(168)*	634 ± 102 ↘ 600 465-1280 *(164)*	**< 0.01**

	Liver			*Kidney*			*Muscle*		
	Beach	Centre	p =	Beach	Centre	p =	Beach	Centre	p =
Cu	52 ± 17 52 14 - 100 *(144)*	51 ± 26 50 10 - 152 *(104)*	ns	28 ±12 27 1.1 - 76.3 *(110)*	31 ±13 31 2 - 74 *(53)*	ns	18 ± 6 18 9 - 53 *(145)*	20 ± 11 18 3 - 90 *(107)*	ns
Zn	145 ±39 138 66 - 328 *(144)*	168 ±46 ↗ 158 84 - 413 *(104)*	**< 0.01**	169 ± 41 168 41 - 284 *(111)*	173 ± 46 176 37 - 286 *(54)*	ns	60 ± 14 59 31 - 131 *(145)*	73 ±31 ↗ 67 36 - 235 *(107)*	**< 0.01**
Fe	2549 ±1354 2274 393 - 5928 *(144)*	3557 ± 1564 ↗ 3468 775 - 7946 *(104)*	**< 0.01**	613 ± 294 569 122 - 2376 *(111)*	700 ± 257 668 367 - 1759 *(53)*	< 0.05	669 ± 241 641 337 - 2428 *(145)*	903 ± 784 ↗ 732 78 - 5724 *(107)*	**< 0.01**
Cd	2.4 ± 1.6 2.1 <dl - 10.1 *(144)*	2.5 ± 1.9 2.1 <dl - 9.7 *(104)*	ns	7.8 ± 6.5 6.4 <dl - 39.9 *(111)*	6.2 ±5.3 4.7 <dl - 30.2 *(54)*	ns	<dl	<dl	

Table 2: body mass (g) and trace elements concentrations (µg/g dw) in liver, kidneys, and muscles of guillemots collected either directly from the beach (Beach) or after a stay in a rehabilitation centre (Centre), expressed as a mean ± standard deviation, median, range of concentration (min-max), and number of samples (n); nd = non determined, <dl = below detection limit, ns = not significant. Total and polar lipids are expressed as g/g dw. Statistical significant differences at $p<0.01$ are shown by plain line boxes.

	Liver			*Kidney*			*Muscle*		
	Beach	Centre	p =	Beach	Centre	p =	Beach	Centre	p =
Total Hg	5.9 ± 2.9 5.4 0.8 - 20.7 *(156)*	7.9 ± 6.3 6.0 1.2 - 35.8 *(125)*	< 0.05	4.6 ± 3.0 4.0 1.0 - 23.8 *(90)*	5.7 ± 2.4 4.5 4.4 - 9.3 *(4)*	ns	2.1 ± 1.1 1.8 0.3 - 6.7 *(163)*	3.8 ± 3.4 2.8 0.4 - 23.2 *(139)*	**< 0.01**
Org. Hg	4.6 ± 2.2 4.1 0.8 - 14.1 *(138)*	6.6 ± 5.4 5.0 1.3 - 32.3 *(105)*	**< 0.01**	3.3 ± 1.5 3.0 1.0 - 6.9 *(55)*	5.2 ± 1.8 4.6 3.6 - 7.8 *(4)*	< 0.05	1.6 ± 0.8 1.4 0.3 - 4.9 *(136)*	3.1 ± 2.8 2.2 0.4 - 17.8 *(114)*	**< 0.01**
Inorg.Hg	1.1 ± 1.1 0.9 0.0 - 6.5 *(135)*	1.3 ± 1.6 0.9 0.0 - 10.8 *(105)*	ns	0.9 ± 0.7 0.7 0.0 - 2.6 *(54)*	0.6 ± 0.7 0.5 0.0 - 1.5 *(4)*	ns	0.4 ± 0.4 0.3 0.0 - 1.9 *(136)*	0.6 ± 0.8 0.4 0.0 - 5.3 *(113)*	ns
Sum PCB	5.7 ± 6.0 3.5 0.3 - 27.2 *(130)*	11.7 ± 13.0 8.7 1.0 - 60.4 *(68)*	**< 0.01**	3.4 ± 2.8 2.6 0.1 - 12.8 *(88)*	2.6 ± 1.6 2.2 1.1 - 4.7 *(4)*	ns	2.1 ± 1.8 1.6 0.1 - 10.5 *(130)*	5.4 ± 10.0 3.0 0.2 - 81.9 *(77)*	**< 0.01**
Total lipids	0.18 ± 0.07 0.17 0.03 - 0.60 *(120)*	0.16 ±0.07 0.15 0.06 - 0.33 *(32)*	ns	nd	nd		0.10 ± 0.08 0.08 0.01 - 0.63 *(119)*	0.14 ± 0.12 0.10 0.05 - 0.65 *(31)*	**< 0.01**
Polar lipids	0.11 ± 0.03 0.11 0.04 - 0.29 *(130)*	0.11 ± 0.04 0.10 0.07 - 0.27 *(68)*	ns	0.12 ± 0.02 0.12 0.03 - 0.17 *(88)*	0.07 ± 0.04 0.08 0.01 - 0.11 *(4)*	< 0.05	0.04 ± 0.03 0.03 0.01 - 0.16 *(130)*	0.05 ± 0.04 0.04 0.01 - 0.19 *(77)*	ns

Table 2 (continued): body mass (g) and trace elements concentrations (µg/g dw) in liver, kidneys, and muscles of guillemots collected either directly from the beach (Beach) or after a stay in a rehabilitation centre (Centre), expressed as a mean ± standard deviation, median, range of concentration (min-max), and number of samples (n); nd = non determined, <dl = below detection limit, ns = not significant. Total and polar lipids are expressed as g/g dw.
Statistical significant differences at p<0.01 are shown by plain line boxes.

We systematically examined the influence of age, sex, the most frequent lesions (cachexia, acute hemorrhagic gastro-enteropathy) and stable pollutant levels (heavy metals and PCBs) on the contamination levels of the tissues (tables 3 a, b, and c). No clear-cut differences appeared between class I (juvenile and immature) v/s class II (adult) birds, nor between male and female birds, except for cadmium concentrations which were twice as high in adult kidney ($p<0.01$). The two groups displayed median Cd concentrations of 4.9 and 9.2 µg/g dw, with different distribution patterns for class I and class II (figure 2). Variations in kidney Cd levels are likely to reflect both dietary differences and age accumulation effects. Cd concentrations in the kidney has been shown to correlate with age in several seabird species (Thompson, 1990; Lock *et al.*, 1992).

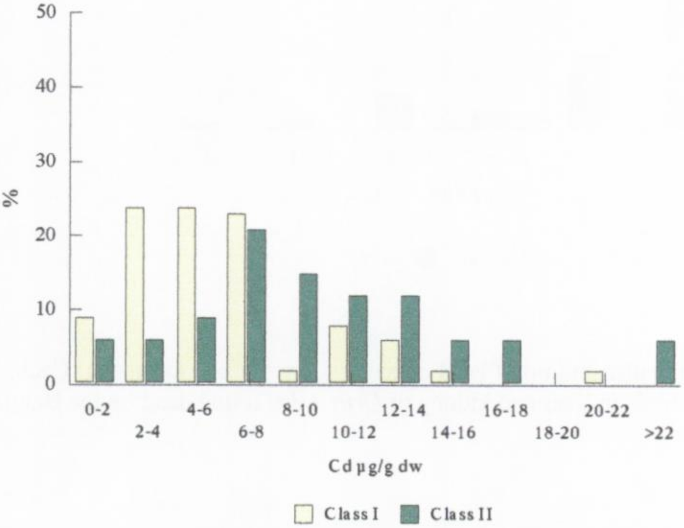
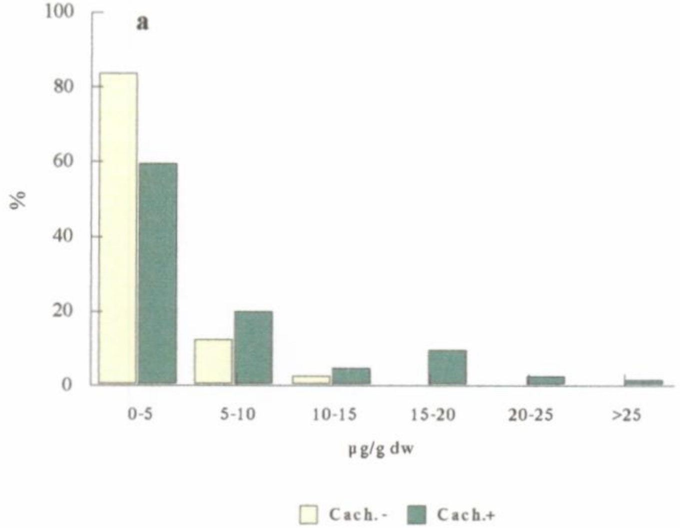


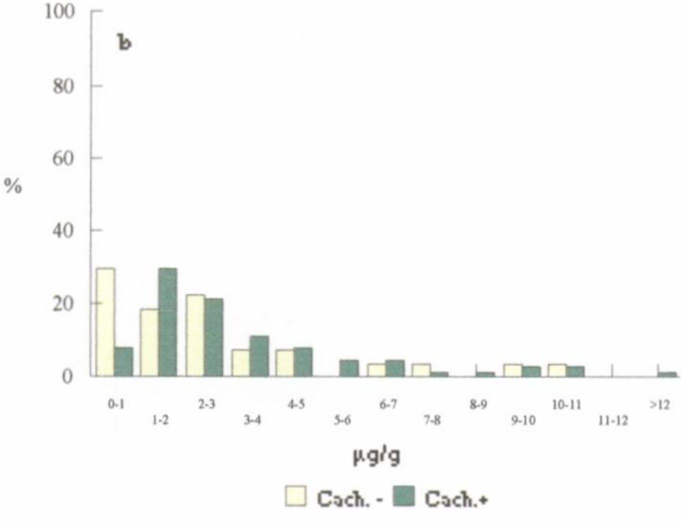
Figure 2: relative distribution of Cd concentration for age class I (juvenile and immature) and age class II (adult) in kidney of *Uria aalge* found dead on the Belgian coast.

One might expect a general increase of pollutant levels in the case of cachexia. Apart from a general decrease of subcutanous fat, the total weight loss in case of cachectic birds (708 ± 116 g, non cachectic 781 ± 140 g) was linked to a general decrease in muscle lipid content. Elevated liver levels for PCBs in the case of cachectic birds might indicate a remobilization after depletion of fat deposits (figures 3a and b). It is also worth noting that the highest levels for PCBs, particularly in liver, were always found in cachectic animals. For all tissues, significantly higher levels of Zn were also linked to the status of cachexia.

Acute hemorrhagic gastro-enteropathy showed no clear relation with levels of stable contaminants, except in case of organic Hg which was found in higher concentrations in the kidney of animals which had developed acute hemorrhagic gastro-enteropathy (table 3 b). The inflammatory nature of the intestinal lesion could not be conclusively assessed, due to the poor quality of the material for histopathology.



Figures 3 a and b: relative distribution of PCB concentrations for non cachectic (Cach.-) and cachectic (Cach.+) birds in liver and kidney of *Uria aalge* found dead on the Belgian coast.



	Age Class I	Class II	Sex		Cachexia		Gastro-enteropathy		Oiling		
	Juv. + imm.	Adult	Male	Female	-	+	-	+	no	E. + I.	E.
Body mass	707 ± 109 688 *(92)*	761 ± 133 725 *(49)*	725 ± 123 700 *(86)*	717 ± 117 700 *(65)*	781 ± 140 760 *(40)*	↘ 708 ± 116 680 *(126)*	748 ± 132 707 *(64)*	711 ± 120 687 *(102)*	698 ± 116 680 *(75)*	745 ± 123 715.0 *(51)*	747 ± 137 700.0 *(41)*
Liver											
Cu	51 ± 17 51 *(75)*	52 ± 17 53 *(42)*	53 ± 17 53 *(74)*	51 ± 16 51 *(54)*	48 ± 20 45 *(36)*	53 ± 16 52 *(108)*	52 ± 17 57 *(55)*	52 ± 17 52 *(89)*	51 ± 16 52 *(66)*	52 ± 16 50 *(39)*	53 ± 21 55 *(38)*
Zn	145 ± 39 137 *(75)*	150 ± 38 147 *(42)*	147 ± 46 134 *(74)*	145 ± 28 143 *(54)*	129 ± 33 125 *(36)*	↗ 151 ± 40 143 *(108)*	148 ± 47 130 *(55)*	144 ± 33 140 *(89)*	142 ± 34 136 *(66)*	150 ± 30 145 *(39)*	147 ± 54 132 *(38)*
Fe	2325 ± 1202 2178 *(75)*	2867 ± 1438 2554 *(42)*	2353 ± 1087 2231 *(74)*	2758 ± 1603 2372 *(54)*	2528 ± 1391 2130 *(36)*	2557 ± 1347 2320 *(108)*	2701 ± 1484 2337 *(55)*	2455 ± 1266 2260 *(89)*	2509 ± 1424 2318 *(66)*	2499 ± 1335 2189 *(39)*	2679 ± 1288 2362 *(38)*
Cd	2.2 ± 1.6 1.9 *(75)*	2.7 ± 1.4 2.3 *(42)*	2.4 ± 1.5 2.2 *(74)*	2.7 ± 1.8 2.2 *(54)*	2.5 ± 2.3 2.0 *(36)*	2.4 ± 1.3 2.2 *(108)*	2.7 ± 2.1 2.3 *(55)*	2.2 ± 1.3 2.0 *(89)*	2.7 ± 2.1 2.1 *(66)*	2.2 ± 1.2 2.0 *(39)*	2.2 ± 1.2 2.1 *(38)*
Total Hg	6.4 ± 3.2 5.7 *(87)*	5.1 ± 2.4 4.7 *(45)*	4.3 ± 2.1 4.1 *(20)*	5.9 ± 2.4 6.0 *(24)*	5.3 ± 2.3 4.9 *(37)*	6.1 ± 3.1 5.5 *(118)*	5.8 ± 2.8 5.5 *(57)*	6.0 ± 3.1 5.3 *(98*	5.3 ± 1.9 5.3 *(71)*	6.9 ± 3.9 6.9 *(43)*	5.7 ± 3.0 5.5 *(41)*
Org. Hg	4.9 ± 2.4 4.2 *(75)*	4.2 ± 2.1 3.9 *(41)*	3.5 ± 1.8 3.0 *(17)*	4.9 ± 2.1 4.8 *(23)*	4.3 ± 2.0 4.3 *(34)*	4.7 ± 2.3 4.0 *(103)*	4.6 ± 2.3 4.2 *(51)*	4.6 ± 2.3 3.9 *(86)*	4.2 ± 1.3 4.1 *(63)*	5.1 ± 2.8 4.3 *(37)*	4.6 ± 2.7 3.9 *(37)*
Inorg. Hg	1.2 ± 1.3 0.9 *(74)*	1.0 ± 1.1 0.7 *(40)*	1.0 ± 1.0 0.7 *(17)*	1.1 ± 1.2 0.5 *(22)*	0.8 ± 0.9 0.6 *(33)*	1.2 ± 1.2 0.9 *(101)*	1.1 ± 1.0 0.8 *(49)*	1.2 ± 1.2 0.9 *(85)*	1.0 ± 0.8 0.9 *(63)*	1.4 ± 1.5 0.8 *(34)*	1.2 ± 1.2 0.7 *(37)*
Sum PCB	5.2 ± 5.8 3.3 *(68)*	7.5 ± 6.8 5.5 *(40)*	4.2 ± 2.6 4.1 *(17)*	10.4 ± 7.8 9.8 *(22)*	2.8 ± 2.9 1.7 *(31)*	↗ 6.6 ± 6.4 4.2 *(99)*	6.0 ± 6.4 3.5 *(46)*	5.6 ± 5.8 3.5 *(84)*	4.8 ± 4.8 3.3 *(68)*	8.1 ± 7.1 5.0 *(22)*	6.0 ± 6.9 3.5 *(39)*
Total lipids	0.17 ± 0.08 0.17 *(58)*	0.18 ± 0.05 0.18 *(35)*	0.17 ± 0.08 0.17 *(60)*	0.18 ± 0.05 0.18 *(44)*	0.18 ± 0.07 0.18 *(32)*	0.18 ± 0.07 0.17 *(88)*	0.19 ± 0.05 0.18 *(43)*	0.17 ± 0.07 0.17 *(77)*	0.18 ± 0.07 0.17 *(66)*	0.18 ± 0.06 0.19 *(17)*	0.18 ± 0.06 0.17 *(36)*
Polar lipids	0.11 ± 0.03 0.11 *(68)*	0.11 ± 0.04 0.10 *(40)*	0.12 ± 0.04 0.12 *(17)*	0.10 ± 0.02 0.1 *(22)*	0.11 ± 0.05 0.1 *(31)*	0.11 ± 0.03 0.11 *(99)*	0.11 ± 0.03 0.10 *(46)*	0.11 ± 0.03 0.11 *(84)*	0.11 ± 0.03 0.11 *(68)*	↘ 0.09 ± 0.02 0.09 *(22)*	0.12 ± 0.04 0.11 *(39)*

Table 3a : body mass (g) and trace elements concentrations (μg/g dw) in liver of guillemots expressed as a mean ± standard deviation, median and number of samples (n) in individuals found dead on the shores (n=170) : class I (juvenile and immature) and class II (adult), male and female, non cachectic (-) and cachectic (+), acute hemorrhagic gastro-enteropathy negative (-) and positive (+), oiling : no oiling, external and internal oiling (E+I) and external only (E). Total and polar lipid contents expressed as g/g dw. Statistical significant differences at p< 0.01 are shown by plain line boxes.

	Age		Sex		Cachexia		Gastro-enteropathy		Oiling		
	Class I	Class II									
	Juv. + imm.	Adult	Male	Female	-	+	-	+	no	E. + I.	E.
Kidneys											
Cu	28 ± 14 27 *(53)*	29 ± 11 29 *(33)*	28 ± 13 27 *(56)*	29 ± 13 29 *(40)*	22 ± 12 18 *(27)*	↗ 30 ± 12 29 *(83)*	27 ± 13 27 *(38)*	28 ± 12 27 *(72)*	29 ± 11 28 *(61)*	25 ± 13 25 *(12)*	27 ± 14 27 *(36)*
Zn	170 ± 42 170 *(53)*	175 ± 40 182 *(33)*	169 ± 42 164 *(56)*	177 ± 39 187 *(40)*	150 ± 46 147 *(28)*	↗ 176 ± 37 177 *(83)*	161 ± 45 160 *(37)*	174 ± 38 173 *(73)*	174 ± 41 176 *(61)*	159 ± 35 155 *(12)*	164 ± 42 167 *(37)*
Fe	597 ± 320 563 *(53)*	622 ± 259 629 *(33)*	610 ± 216 594 *(56)*	592 ± 358 559 *(40)*	748 ± 278 694 *(28)*	↘ 567 ± 287 540 *(83)*	617 ± 270 604 *(37)*	611 ± 307 563 *(73)*	542 ± 225 529 *(61)*	688 ± 259 689 *(12)*	702 ± 375 650 *(37)*
Cd	5.9 ± 4.0 4.9 *(53)*	↗ 10.4 ± 6.8 9.2 *(33)*	7.2 ± 6.2 6.1 *(56)*	9.4 ± 7.3 7.1 *(40)*	9.2 ± 10.0 5.9 *(28)*	7.3 ± 4.9 6.5 *(83)*	8.6 ± 8.1 6.5 *(38)*	7.3 ± 5.6 6.3 *(73)*	8.3 ± 7.5 6.3 *(61)*	4.4 ± 2.5 3.8 *(12)*	7.9 ± 5.6 6.8 *(37)*
Total Hg	4.9 ± 3.5 4.0 *(45)*	4.3 ± 2.6 3.5 *(24)*	3.9 ± 2.1 3.6 *(12)*	5.1 ± 3.0 3.8 *(11)*	4.0 ± 1.9 3.5 *(26)*	4.8 ± 3.2 4.1 *(64)*	4.0 ± 2.2 3.5 *(32)*	4.9 ± 3.2 4.2 *(58)*	4.0 ± 1.5 4.0 *(49)*	↗ 8.7 ± 6.9 7.2 *(7)*	4.5 ± 2.6 3.5 *(33)*
Org. Hg	3.6 ± 1.5 3.1 *(25)*	2.9 ± 1.6 2.5 *(16)*	2.9 ± 1.3 2.5 *(9)*	3.1 ± 2.0 2.5 *(6)*	2.9 ± 1.5 2.4 *(19)*	3.5 ± 1.4 3.3 *(36)*	2.6 ± 0.9 2.4 (18)	↗ 3.6 ± 1.6 3.7 (37)	3.0 ± 1.0 3.0 *(33)*	4.9 ± 1.7 5.9 *(3)*	3.5 ± 1.8 3.0 *(18)*
Inorg. Hg	0.8 ± 0.7 0.7 *(25)*	0.9 ± 0.8 0.6 *(15)*	0.8 ± 0.9 0.5 *(8)*	1.1 ± 0.7 1.0 *(6)*	0.8 ± 0.6 0.7 *(18)*	0.9 ± 0.7 0.8 *(36)*	0.8 ± 0.6 0.8 *(18)*	0.9 ± 0.7 0.7 *(36)*	0.8 ± 0.6 0.8 *(32)*	1.2 ± 0.8 0.9 *(3)*	0.9 ± 0.8 0.7 *(18)*
Sum PCB	3.0 ± 2.4 2.4 *(43)*	3.3 ± 3.1 2.8 *(25*	2.6 ± 1.9 2.8 *(13)*	4.5 ± 3.9 3.4 *(11)*	2.9 ± 2.8 2.1 *(27)*	3.6 ± 2.8 2.8 *(61)*	3.6 ± 3.1 2.9 *(32)*	3.3 ± 2.7 2.6 *(56)*	3.2 ± 2.7 2.5 *(46)*	4.9 ± 4.4 2.8 *(7)*	3.2 ± 2.7 2.8 *(34)*
Total lipids	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
Polar lipids	0.12 ± 0.02 0.12 *(43)*	0.12 ± 0.03 0.13 *(25)*	0.12 ± 0.03 0.13 *(13)*	0.11 ± 0.03 0.11 *(11)*	0.11 ± 0.02 0.11 *(27)*	0.12 ± 0.02 0.12 *(61)*	0.11 ± 0.02 0.12 *(32)*	0.12 ± 0.02 0.12 *(56)*	0.12 ± 0.2 0.12 *(46)*	0.11 ± 0.03 0.11 *(7)*	0.12 ± 0.02 0.12 *(34)*

Table 3b : body mass (g) and trace elements concentrations (µg/g dw) in kidneys of guillemots expressed as a mean ± standard deviation, median and number of samples (n) in individuals found dead on the shores (n=170) : class I (juvenile and immature) and class II (adult), male and female, non cachectic (-) and cachectic (+), acute hemorrhagic gastro-enteropathy negative (-) and positive (+), oiling : no oiling, external and internal oiling (E+I) and external only (E). Total and polar lipid contents expressed as g/g dw. Statistical significant differences at $p < 0.01$ are shown by plain line boxes.

	Age Class I	Class II	Sex		Cachexia		Gastro-enteropathy		Oiling		
	Juv. + imm.	Adult	Male	Female	-	+	-	+	no	E. + I.	E.
Muscle											
Cu	18 ± 6 18 *(77)*	16 ± 4 16 *(41)*	18 ± 6 18 *(76)*	18 ± 5 17 *(53)*	18 ± 5 18 *(37)*	18 ± 6 18 *(108)*	17 ± 5 17 *(56)*	19 ± 6 18 *(89)*	20 ± 7 19 *(65)*	16 ± 4 16 *(41)*	18 ± 5 18 *(38)*
Zn	61 ± 15 59 *(77)*	58 ± 11 57 *(41)*	58 ± 16 55 *(76)*	61 ± 11 61 *(53)*	53 ± 12 52 *(37)*	62 ± 14 62 *(108)*	57 ± 13 55 *(56)*	62 ± 15 61 *(89)*	63 ± 15 63 *(65)*	56 ± 11 54 *(41)*	59 ± 15 55 *(38)*
Fe	693 ± 292 643 *(77)*	612 ± 130 584 *(41)*	688 ± 297 641 *(76)*	649 ± 131 640 *(53)*	586 ± 145 580 *(37)*	697 ± 260 645 *(108)*	646 ± 238 599 *(56)*	683 ± 242 663 *(89)*	711 ± 264 663 *(65)*	601 ± 107 586 *(41)*	674 ± 289 648 *(38)*
Cd	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
Total Hg	2.2 ± 1.1 1.9 *(89)*	2.0 ± 1.2 1.7 *(49)*	1.6 ± 0.9 1.4 *(20)*	2.3 ± 1.4 1.9 *(28)*	2.0 ± 0.8 2.1 *(39)*	2.1 ± 1.2 1.7 *(122)*	2.2 ± 1.3 2.0 *(62)*	2.0 ± 1.0 1.7 *(99)*	1.9 ± 0.8 1.8 *(72)*	2.5 ± 1.3 2.3 *(49)*	2.0 ± 1.3 1.5 *(41)*
Org. Hg	1.7 ± 0.8 1.6 *(77)*	1.5 ± 0.9 1.3 *(39)*	1.2 ± 0.8 1.0 *(15)*	1.7 ± 0.9 1.4 *(23)*	1.5 ± 0.7 1.5 *(33)*	1.7 ± 0.9 1.4 *(101)*	1.7 ± 1.0 1.4 *(49)*	1.6 ± 0.8 1.4 *(85)*	1.5 ± 0.6 1.4 *(64)*	1.8 ± 0.9 1.5 *(35)*	1.6 ± 1.1 1.3 *(36)*
Inorg. Hg	0.4 ± 0.4 0.3 *(77)*	0.4 ± 0.4 0.2 *(39)*	0.3 ± 0.3 0.2 *(15)*	0.4 ± 0.5 0.3 *(23)*	0.4 ± 0.4 0.3 *(33)*	0.4 ± 0.4 0.3 *(101)*	0.4 ± 0.4 0.3 *(49)*	0.4 ± 0.4 0.3 *(85)*	0.3 ± 0.4 0.2 *(64)*	0.6 ± 0.4 0.5 *(35)*	0.3 ± 0.4 0.2 *(36)*
Sum PCB	1.8 ± 1.4 1.3 *(68)*	2.6 ± 2.1 2.1 *(40)*	1.9 ± 0.9 1.6 *(17)*	3.1 ± 2.5 2.5 *(22)*	2.5 ± 2.3 2.0 *(31)*	2.0 ± 1.5 1.6 *(99)*	2.4 ± 1.9 1.9 *(46)*	2.0 ± 1.7 1.6 *(84)*	1.7 ± 1.4 1.3 *(68)*	2.9 ± 2.5 2.0 *(22)*	2.4 ± 1.7 2.0 *(39)*
Total lipids	0.08 ± 0.04 0.08 *(59)*	0.11 ± 0.08 0.08 *(33)*	0.11 ± 0.09 0.08 *(61)*	0.09 ± 0.05 0.08 *(42)*	0.13 ± 0.11 0.11 *(32)*	0.09 ± 0.05 0.08 *(87)*	0.12 ± 0.11 0.08 *(43)*	0.09 ± 0.04 0.08 *(76)*	0.09 ± 0.05 0.08 *(65)*	0.09 ± 0.05 0.07 *(17)*	0.13 ± 0.11 0.10 *(36)*
Polar lipids	0.04 ± 0.02 0.03 *(68)*	0.05 ± 0.04 0.04 *(40)*	0.06 ± 0.033 0.04 *(17)*	0.04 ± 0.03 0.03 *(22)*	0.07 ± 0.04 0.05 *(31)*	0.04 ± 0.01 0.03 *(99)*	0.05 ± 0.04 0.04 *(46)*	0.04 ± 0.02 0.03 *(84)*	0.03 ± 0.01 0.03 *(68)*	0.05 ± 0.03 0.04 *(22)*	0.05 ± 0.04 0.04 *(39)*

Table 3c : body mass (g) and trace elements concentrations (µg/g dw) in pectoral muscles of guillemots expressed as a mean ± standard deviation, median and number of samples (n) in individuals found dead on the shores (n=170) : class I (juvenile and immature) and class II (adult), male and female, non cachectic (-) and cachectic (+), acute hemorrhagic gastro-enteropathy negative (-) and positive (+), oiling : no oiling, external and internal oiling (E+I) and external only (E). Total and polar lipid contents expressed as g/g dw. Statistical significant differences at p< 0.01 are shown by plain line boxes.

		Time	Place	Cu	Zn	Cd	Total Hg	
Liver	n = 51	1970-1981	Belgian coast	nd	nd	nd	7.2 ± 2.4	Delbeke et al., 1984
	n = 83	April to Nov. 1988	Northwest Scotland	range 12.9 - 16.1	range 58.4 - 69.7	range 1.4 - 2.5	range 0.9 - 3.7	Stewart et al., 1994
	n = 10	summer 1992 and 1993	Hornoya North. Norway	20.0 ± 2.9	86.7 ± 14.9	3.1 ± 1.1	1.9 ± 0.4	Wenzel & Gabrielsen, 1994
	n = 66	***winter 1989 to 95***	***Belgian coast***	***51 ± 16***	***142 ± 34***	***2.6 ± 2.0***	***5.3 ± 1.9***	***this study***
Kidneys	n = 9	1970-1981	Belgian coast	nd	nd	nd	4.4 ± 1.7	Delbeke et al., 1984
	n = 10	summer 1992 and 1993	Hornoya North. Norway	14.4 ± 1.9	114 ± 13	24.1 ± 7.5	1.5 ± 0.2	Wenzel & Gabrielsen, 1994
	n = 83	April to Nov. 1988	Northwest Scotland	range 12.3 - 15.2	range 59.3 - 74.1	range 1.6 - 11.7	range 0.8 - 3.9	Stewart et al., 1994
	n = 61	***winter 1989 to 95***	***Belgian coast***	***29 ± 11***	***174 ± 41***	***8.2 ± 7.5***	***4.0 ± 1.5***	***this study***
Muscle	n = 24	April to Nov. 1988	Northwest Scotland	range 10.2 - 14.0	range 20.9 - 26.0	nd	range 0.5 - 1.8	Stewart et al., 1994
	n = 10	summer 1992 and 1993	Hornoya North. Norway	19.2 ± 0.9	49.3 ± 3.3	0.2 ± 0.1	0.4 ± 0.1	Wenzel & Gabrielsen, 1994
	n =	***winter 1989 to 95***	***Belgian coast***	***20 ± 7***	***63 ± 15***	***<dl***	***1.9 ± 0.8***	***this study***

Table 4: comparison of trace elements concentrations (µg/g dw), expressed as a range of mean values or as a mean ± standard deviation, in guillemots of different origins. Data from the Belgian coast shown in plain line boxes. nd: non determined, <dl: below detection limit.

However, we felt the lesion was worth mentioning, since it affected 61 % of the birds and had no clear correlation with decay. Previous reports mentioned a hemorrhagic gastro-enteritis as a terminal lesion, stress related, in marine birds (Dorrestein & van der Hage, 1993; Leighton, 1993). In addition, parasitological and bacteriological examinations failed to isolate a likely infectious cause for that lesion (Jauniaux & Coignoul, 1994; Jauniaux *et al.*, 1996; Brosens *et al.*, 1996). No significant overall trend could be linked to the status of oiling when comparing non-oiled and externally oiled birds, which could partially be explained by the fact that external oiling may have occured as a postmortem artifact. However, significant differences in metal content appeared at different levels when comparing non-oiled guillemots with individuals which were oiled both externally and internally; it is yet unclear whether or not these differences can be linked to changes in the metabolisation of the involved metals in response to oiling.

Compared to guillemots captured in the northern Norway area (Wenzel & Gabrielsen, 1995) and to those shot in northwest Scotland (Stewart *et al.*, 1994), the individuals collected on the Belgian coast were heavily contaminated with Cu, Zn and Hg (table 4). Similar high Cu and Zn levels for *Uria aalge* and for other species from the Belgian coast *(Larus ridibundus, Rissa tridactyla, Melanitta nigra)* were described by Antoine and co-workers (1992) and Bouquegneau and co-workers (1994). Moreover, a previous study on the speciation of metals in the cytosol of the liver and kidney of *Uria aalge* stranded along the Belgian coast showed that the birds failed to maintain constant Cu, Zn and Cd levels on the high molecular weight soluble proteins in both organs; only a small part of the metal in excess was found to be detoxified by metallothioneins (Bouquegneau *et al.*, 1996).

Conclusions

Oiling is a major cause of death for wintering guillemots in the southern North Sea: 55 % of guillemots found on the Belgian shores showed evidence of external or internal oiling. However, a large majority of birds (76 %) were in a state of cachexia probably due to unavailibility of food, bad weather conditions and natural disease. On the other hand, high levels of Cu, Zn, Hg and PCBs were clearly linked to cachexia, which can be considered as favourable to the development of lethal, acute, hemorrhagic gastro-enteropathy. None of these pollutants can be considered as the unique and direct cause of death, but might be an additional physiological stress, leading to debilitation and death. Further research is needed to determine the actual effects of stable pollutants on the health status of guillemots. The beaching of birds can be considered as a multifactorial response to numerous natural phenomena and a series of anthropogenic threats.

Acknowledgements

This study was financed by the 'Impulse Programme in Marine Sciences supported by the Belgian state - The Prime Minister's Services - Scientific, Technical and Cultural Affairs.' (MS/03/31, MS/12/032 and MS/12/033), by the 'European Commission' (13M/91/138, 1366-188901) and by The French Community of Belgium (nr 89/94-131). The authors wish to thank P. Meire, H. Offringa, J. Seys, W. Roggeman, J. Tavernier and the rehabilitation centres of Oostende, Nieuwpoort, Blankenberge and Het Zwin, for providing the specimens, but also R. Biondo and M. Bossicart for their valuable technical assistance. We also wish to thank Professor A. Distèche for critical reading of the manuscript and M. Doyen for his assistance to sort and process raw data for statistical analyses.

References

ANTOINE N., JANSEGERS I., HOLSBEEK L., JOIRIS C., BOUQUEGNEAU, J.-M. (1992). Contamination par les métaux lourds des oiseaux marins et des marsouins de la Mer du Nord. Bulletin de la Société Royale des Sciences de Liège 61: 163-176.

BARNES H. & BLACKSTOCK J. (1973). Estimation of lipids in marine animals and tissues: detailed investigation of the sulphosphovanillin method for 'total' lipids. Journal of Experimental Marine Biology and Ecology 12: 103-118.

BIRKHEAD, T.R. (1974). Movement and mortality rates of British guillemots. Bird Study 21: 241-254.

BOUQUEGNEAU, J.-M. (1973). Etude de l'intoxication par le mercure d'un poisson téléostéen *Anguilla anguilla*. I Accumulation du mercure dans les organes. Bulletin de la Société Royale des Sciences de Liège 9-10: 440-446.

BOUQUEGNEAU, J.-M., DEBACKER, V., ANTOINE, N., COIGNOUL, F., HOLSBEEK, L., JAUNIAUX, T., TAPIA, G., JOIRIS, C. (1994). Causes of mortality and heavy metals content of guillemots *Uria aalge* stranded along the Belgian Coast. Bulletin de la Société Royale des Sciences de Liège 63: 211-217.

BOUQUEGNEAU, J.M., DEBACKER, V., GOBERT, S. HAVELANGE, S. (1996). Role of metallothioneins in metal regulation by the guillemot *Uria aalge*. Comparative Biochemistry and Physiology 113 C: 135-139.

BOURNE, W.R.P. & VAUK, G. (1988). Human impact upon North Sea birds. In: Salomons W., Bayne B.L., DUURSMA, E.K., FORSTNER, U. (Eds). Pollution of the North Sea - an assessment. Springer-Verlag, Heidelberg, 579-595.

BROSENS L., JAUNIAUX T., SIEBERT U., BENKE H., COIGNOUL F. (1996). Observations of the helminths of harbour porpoises (*Phocoena phocoena*) and common guillemots (*Uria aalge*) from the Belgian and German coasts. Veterinary Record 139: 254-257.

BURGER, J. & GOCHFELD, M. (1995). Heavy metal 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.

CAMPHUYSEN, C.J. & VAN FRANEKER, J.A. (1992). The value of beached bird surveys in monitoring marine oil pollution. Techn. Rapp. Vogelbescherming 10. Vogelbescherming Nederland, Zeist, 191 pp.

CAMPHUYSEN, C.J. & LEOPOLD, M.F. (1994). Atlas of seabirds in the southern North Sea. IBN Research Report 94/6, NIOZ-Report 1994-8, Institute for Forestry and Nature Research, Dutch Seabird Group and Netherlands Institute for Sea Research, Texel, 126 pp.

CAMPHUYSEN, C.J. (1995). Beached birds in the Netherlands as indicators of marine oil pollution. Sula 9: 86-90.

CARTER, I.C., WILLIAMS, J.M., WEBB, A., TASKER, M.L. (1993). Seabird concentrations in the North Sea: an atlas of vulnerability to surface pollutants. Joint Nature Conservation Committee, Aberdeen.

DAHLMANN, G., TIMM, D., AVERBECK, C.H.R., CAMPHUYSEN, C., SKOV, H. DURINCK, J. (1994). Oiled seabirds- comparative investigations on oiled seabirds and oiled beaches in the Netherlands, Denmark and Germany (1990-93). Marine Pollution Bulletin 28: 305-310.

DELBEKE, K, JOIRIS, C., DECADT, G. (1984). Mercury contamination of the Belgian avifauna, 1970-1981. Environmental Pollution Serie B 7: 205-221.

DORRESTEIN, G.M. & VAN DER HAGE, M.H. (1993). Diagnostic pathology. Proceedings of the European Conference of Avian Medecine and Surgery, Utrecht.

DORRESTEIN G.M. & VAN DER HAGE M.H. (1996). Marine bird necropsy findings. In: Jauniaux, T., Bouquegneau, J.-M., Coignoul, F. (Eds). Marine mammals, seabirds, and pollution of marine systems. Presses de la Faculté de médecine Vétérinaire de l'Univrsité de Liège, 151-166.

DUNNET, G.M., FURNESS, R.W., TASKER, M.L., BECKER, P.H. (1990). Seabird ecology in the North Sea. Netherland Journal of Sea Research 26: 387-425.

ELLIOT, J.E., SCHEUHAMMER A.M., LEIGHTON, F.A., PEARCE, P.A. (1992). Heavy metal and metallothionein concentrations in Atlantic Canadian seabirds. Archives of Environmental Contamination and Toxicology 22: 63-73.

HATCH, R.W. & OTT, W.L. (1968). Determination of sub-microgram quantities of mercury by atomic absorption spectrophotometry. Analytical Chemistry 40: 2085-2087.

JAUNIAUX, T. & COIGNOUL, F. (1994). Aspergillose chez les oiseaux marins échoués à la côte belge. Annales de Médecine Vétérinaire 4: 59-63.

JAUNIAUX, T., BROSENS, L., FARNIR, F., MANTECA, C., LOSSON, B., TAVERNIER, J., VINDEVOGEL, H., COIGNOUL, F. (1996). Mortalité des oiseaux marins lors de l'hiver 1992-1993 le long du littoral belge. Annales de Médecine Vétérinaire 140: 149-159.

KUYKEN, E. (1978). Beached bird surveys in Belgium. Ibis 120: 122-123.

LANDSBOROUGH (1953). The migration of British Auk (Alcidae) as shown by the results of marking. British Bird XLVI: 3-15.

LEIGHTON, F.A. (1993). The toxicity of oil petroleum to birds. Environmental Review 1: 92-103.

LLOYD, C., TASKER, M.L., PARTRIDGE, K. (1991). The status of breeding seabirds in Britain and Ireland. T. & A.D. Poyser Ltd. London, 355 pp.

LOCK, J.W., THOMPSON, D.R., FURNESS R.W., BARTLE, J.A. (1992). Metal concentrations in seabirds of the New Zealand region. Environmental Pollution 75: 289-300.

MEAD, C.J. (1974). The results of ringing Auks in Britain and Ireland. Bird Study 21: 45-87.

MEAD, C., BAILLIE, S. (1981). Seabirds and oil: the worst winter. Nature 292: 10-11.

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.

NETTLESHIP, D.N. & EVANS, P.G.H. (1985). Distribution and status of the Alcidae. In: Nettleship, D.N. & Birkhead, T.R. (Eds). The Atlantic Alcidae: the evolution, distribution and biology of the Auks inhabiting the Atlantic Ocean and adjacent water areas. Academic Press, London, 84-88.

NORTH SEA TASK FORCE REPORT (1993a). North Sea Subregion 4: assessment report (Netherlands, Belgium, France, Germany and United Kingdom). Oslo and Paris Commission (Olsen & Olsen, eds), 195 pp.

NORTH SEA TASK FORCE REPORT (1993b). North Sea quality status report. Oslo and Paris Commission (Olsen & Olsen, eds), 132 pp.

OHLENDORF, H.M. & FLEMING, W.J. (1988). Birds and environmental contaminants in San Francisco and Chesapeake Bays. Marine Pollution Bulletin 19: 487-495.

PEAKALL, D. (1992). Animals biomarkers as pollution indicators. Chapman & Hall Ecotoxicology Series, 291 pp.

PIONNEAU, F. (1987). Etudes des oiseaux marins échoués. Le Point Vétérinaire 19: 469-478.

SCHEUHAMMER, A.M. (1987). The chronic toxicity of aluminium, cadmium, mercury, and lead in birds: a review. Environmental Pollution 46: 263-295.

STEWART, F.M., THOMPSON, D.R., FURNESS, R.W., HARRISSON, 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.

STOWE, T.J. & UNDERWOOD, L.A. (1984). Oil spillages affecting seabirds in the United Kingdom, 1966-83. Marine Pollution Bulletin 15: 147-152.

THOMPSON, D.R. (1990). Metal levels in marine vertebrates. In: Furness, R.W. & Rainbow, P.S. (Eds). Heavy metals in the marine Environment. CRC Press, London, 143-181.

THOMPSON, D.R., FURNESS, R.W., BARRETT, T. (1992). Mercury concentrations in seabirds from colonies in the Northeast Atlantic. Archives of Environmental Contamination and Toxicology 23: 383-389.

UTHE J.F., SOLOMON J., GRIFT, B. (1972). Metals and other elements. Rapid semimicro method for the determination of methylmercury in fish tissue. Journal of the Association of official analytical chemists 55: 583-589.

WALSH, P.M. (1990) The use of seabirds as monitors of heavy metals in the marine environment. In: Furness, R.W. & Rainbow, P.S. (Eds). Heavy metals in the marine Environment. CRC Press, London, 183-204.

WENZEL, C. & GABRIELSEN, G.W. (1995). Trace element accumulation in three seabird species from Hornφya, Norway. Archives of Environmental Contamination and Toxicology 29: 198-206.

Chapter 3

Heavy metals contamination and body condition of wintering guillemots (*Uria aalge*) at the Belgian coast from 1993 to 1998.

Based on the manuscript by: V. Debacker[1], T. Jauniaux[2], F. Coignoul[2], J.M. Bouquegneau[1]
[1].Oceanology, University of Liège, B6c Sart Tilman, B-4000, Belgium.
[2]. Pathology Department of the Veterinary College, University of Liège, B43 Sart Tilman, B-4000, Belgium.

Environmental Research Section A84 (2000), in press.

Contents

Heavy metals contamination and body condition of wintering guillemots (*Uria aalge*) at the Belgian coast from 1993 to 1998.

Abstract

A sample of 166 common guillemots (*Uria aalge*) recovered from Belgian beaches during five wintering seasons, from 1993-94 to 1997-98, were examined. At necropsy, postmortem examination including body mass, fat reserves, presence or not of intestinal contents, eventual status of oiling and pathological changes (cachexia, **a**cute **h**emorrhagic **g**astro-**e**nteropathy - GEAH -) was attributed to each individual. Mild to severe cachexia, a pathology characterized by moderate to severe atrophy of the pectoral muscle as well as reduced amounts or absence of subcutaneous and/or abdominal fat, was observed for most specimens (85.8 %). Heavy metals analysis (Cu, Zn, Fe, Cd, Ni, Cr and Pb) of the tissues (typically liver, kidneys and pectoral muscles) were performed, and total lipids were determined (liver and pectoral muscles). The guillemots collected at the Belgian coast exhibited higher Cu and Zn concentrations compared to individuals collected in more preserved areas of the North Sea such as the northern colonies. A general decrease of their total body mass as well as liver, kidneys and pectoral muscle mass was associated to increasing cachexia severity. Moreover, significantly increasing heavy metal levels (Cu and Zn) in the tissues as well as depleted muscle lipid contents were observed parallel to increasing cachexia severity. On the contrary, the organs' total metal burdens barely correlates to this status. These observations tend to indicate a general re-distribution of heavy metals within the organs as a result of prolonged starvation and protein catabolism (cachectic status). Such a re-distribution could well be an additional stress to birds already experiencing stressful conditions (starvation, oiling, …).

Introduction

Seasonal changes in weight or body condition are documented for several seabirds species: herring gulls (*Larus argentatus*) (Coulson *et al.*, 1983), fulmars (*Fulmarus glacialis*), Manx shearwater (*Puffinus puffinus*), puffins (*Fratercula arctica*) (Osborn & Harris, 1984) and auks (*Uria* spp. and *Alca torda*) (Barrett *et al.*, 1989; Harris & Wanless, 1988; Furness *et al.*, 1994). Body condition of stranded seabirds, among which the common guillemot *Uria aalge*, often underlines severe emaciation of these animals, with little or no trace of subcutaneous fat deposits and very reduced pectoral muscle mass. A loss of 25 up to 40 % of the body weight compared to normal, healthy populations at sea is not unusual (Hope Jones *et al.*, 1984; Osborn *et al.*, 1984; Camphuysen, 1989; Piatt & Van Pelt, 1997; Jauniaux *et al.*, 1998). However, experimentally fasted guillemots lost up to 20 % of their body weight after a short term fast of 3 days, with no drastic decrease in glucose plasma levels, indicating a resistance to short term fast (Herzberg, 1991). Tolerance to food deprivation is of prime importance to birds of prey, like the guillemot, which may face, especially during the wintering season, difficulties to feed. This starvation process is slow to establish and once the supply of adipose tissue is depleted, proteolysis begins. The breakdown of proteins is the last process to supply energy for life (Huether & McCance, 1994).

Guillemots at the Belgian coast typically strand from mid-November to late March, during their wintering season in the southern North Sea. At necropsy, emaciation, referred to as cachexia (a pathology characterized by a moderate to severe atrophy of the pectoral muscles with reduced amounts or absence of subcutaneous and/or abdominal fat), is the main lesion observed (Jauniaux *et al.*, 1998). In a previous paper Debacker and co-workers (1997), have shown that the cachectic status is associated with significantly higher heavy metals concentrations. At that stage, no discrimination was made between cachectic birds and they were simply opposed to the non cachectic individuals. In the present study covering five successive wintering seasons (from winter 1993-94 to winter 1997-98) data are considered following the cachectic status of the individuals: non cachectic (cach. -) with or without fat deposits and cachectic, respectively cach. +1, cach. +2, cach. +3, with increasing severity. Other relevant parameters (sex, age, **a**cute **h**emorrhagic **g**astro-**e**nteropathy - GEAH - and oiling) are considered. Increasing heavy metal levels are described with increasing cachexia severity. On the contrary, the organs' total metal burdens are not affected by the cachectic status, probably reflecting a general re-distribution of heavy metals as a result of protein catabolism. Increased organochlorines and mercury levels have also been reported for guillemots during their wintering in the southern North Sea (Joiris *et al.*, 1997; Tapia, 1998). A general model describing heavy metals concentrations and total loads modifications (considered as an additional stress) as well as manifest debilitating processes are presented and discussed.

Materials and methods

Collection and storage

A total of 166 guillemots, collected stranded between November to March, during 5 successive wintering periods (from winter 1993-94 to winter 1997-98) were available for toxicological investigations. All were found dead on the beaches. Half of them presented oil residues on the plumage but none had been cleaned prior to collection. Fresh carcasses were necropsied according to a standardized protocol (Jauniaux *et al.*, 1996), while putrefied specimens were discarded. Two age categories were considered as the individuals were aged as juveniles (<1 year old) or adult (potential but not necessarily breeders) based on the presence of the cloacal bursa (Camphuysen & van Franeker, 1992). Sex, total body weight, liver and kidney weight, as well as the presence of oil, intestinal contents, GEAH and other lesions were recorded. An exact determination of the age and/or sex could not be made for some individuals due to partial degradation of the tissues. Emaciation (cachexia) was evaluated using visible signs - presence or absence of subcutaneous fat, light to severe atrophy of the pectoral muscles - and given a range from 1 to 3, depending on its severity: specifically, 1: absence of fat and slight pectoral muscle atrophy; 2: moderate pectoral muscle atrophy; 3: severe pectoral muscle atrophy. Organs were collected (typically liver, kidney and pectoral muscle), weighed and kept frozen (-18° C) prior to toxicological analyses. A condition index was calculated using the liver to kidney weight ratio, as proposed by Wenzel & Adelung (1996). Total load of contaminants for liver, kidneys and pectoral muscles were calculated using dry weight concentrations as well as the organ's water content for each individual.

Heavy metals

Atomic absorption spectrophotometry (ARL 3510) was used to determine heavy metals concentrations (Cu, Zn, Cd, Fe). Pb, Ni, Cr and Ti contents were also determined but the results most often were below the detection limits and will not be discussed here. After being weighed and dried during 48 hours at 110°C, samples were digested with a mixed solution of chloric and nitric acids (1:3; v:v) and slowly heated to 100°C until complete digestion. The samples were then diluted, filtered and analysed. In addition, quality of the analyses was controlled through participation to an intercalibration program (Quevauviller, 1997). Recoveries ranged from 97 to

100 % for Cu and Zn respectively, and 102 % for Cd. A set of certified material (DORM-2, National Research Council, Canada) gave a recovery rate of 92 % for Fe. Limits of detection were 0.18, 0.17, 0.16 and 0.18 $\mu g\ g^{-1}$ dw for Cu, Zn, Fe and Cd, respectively.

Lipids

Lipids were extracted in liver and muscle tissues using a solvent mixture (Folch *et al.*, 1957) of methanol/chloroform (2:1, v:v) followed by purification of the extracts with a KCl solution and several dehydration steps using methanol and absolute ethanol, as described by Barnes & Blackstock (1973).

Data analysis

Statistical analysis of the data was performed using SAS statistical package (SAS Institute, Cary, NC, USA, version 6.12, 1996). Kolmogorov-Smirnov test were used to check if the variables fitted a normal distribution. When not distributed normally the variables were log-transformed to normalize their distribution. Multiple regression analysis considering, for each individual, its age, sex, cachectic status, presence or not of oil and presence or not of GEAH, was used. Results were considered to be significant at the 5 % critical level ($p< 0.05$) and highly significant at the 1 % critical level ($p < 0.01$).

Results and discussion

Body condition

Among the 166 stranded guillemots analysed, collected between late November to late March during five successive wintering seasons, pathological findings point towards a generalized cachectic status with 85.8 % of the birds emaciated to varying degrees (table 1).

	Age			Sex			Intestinal contents				Cachexia				GEAH	
	Juven	Adult	nd	Male	Fem	nd	Norm	Blood	Empty	nd	-	+1	+2	+3	-	+
n	97	55	14	88	61	17	20	89	55	2	27	31	79	29	43	123
%	58.4	33.1	8.4	53	36.7	10.2	12	53.6	33.1	1.2	16.2	18.7	47.6	17.5	25.9	74.1

Table 1: sample size (n) and percentage of juvenile and adult, male and female, non cachectic (-) and cachectic with increasing severity (+1 to +3) and acute hemorrhagic gastro-enteropathy (GEAH) presence (+) or absence (-), of common guillemots collected on Belgian beaches from winter 1993-94 to winter 1997-98. Not determined: n.d.

Statistical analysis did not reveal any correlations between the frequency of the cachectic status observed and sex, age, wintering months considered, presence or absence of oil, or presence or absence of GEAH (all p=ns). For instance, the proportion of cachectic birds does not differ significantly ($p = 0.64$) from month to month throughout the winters (figure 1) with 60 to 100 % of cachectic birds collected each month.

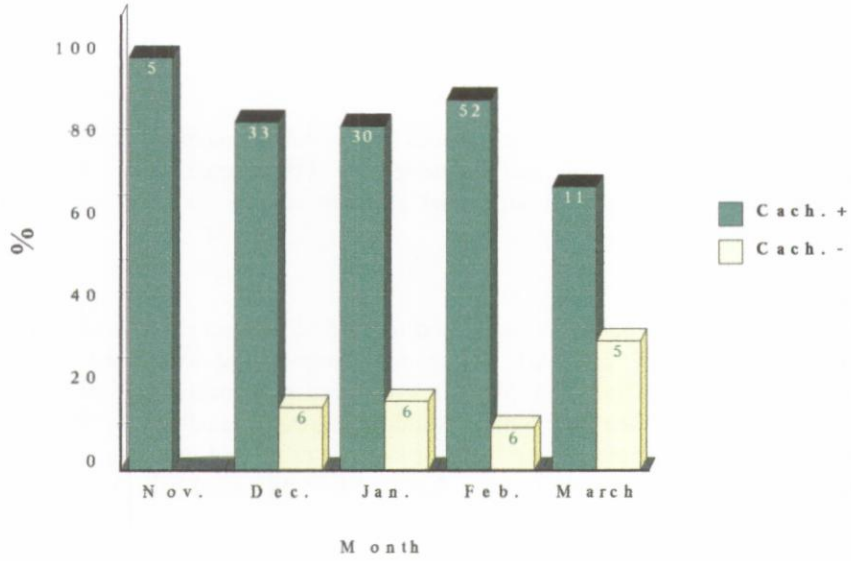


Figure 1: proportion of cachectic guillemots collected during the winter months (November to March), from 1993-94 to 1997-98.

Birds considered as non cachectic in this study have lost 24 % of their normal weight compared to healthier birds weighed at colonies. Furthermore, emaciated individuals have lost 29, 36 and 43 % of their weight (table 2) with increasing cachexia severity (1 to 3) respectively, compared to guillemots weighed at colonies (± 1000 g.: Bédart, 1985; Harris & Wanless, 1988; Furness *et al.*, 1994). These weight losses are not comparable to normal seasonal weight variations and are clearly associated with a severely reduced muscle mass and absence of subcutaneous and abdominal fat, two features of the cachectic status.

Organ	Cachexia - n = 23	 +1 n = 31	 +2 n = 79	 +3 n = 29
Body and organ weight g.				
Body	758 ± 114 *760*	710 ± 85 *690*	640 ± 64 *640*	574 ± 47.3 *570*
Liver	24.9 ± 8.6 *23.3*	19.0 ± 3.7 *18.3*	17.6 ± 3.8 *17.6*	15.9 ± 4.4 *15.0*
Kidneys	10.3 ± 2.4 *10.3*	9.9 ± 1.7 *9.7*	9.2 ± 1.2 *9.2*	8.3 ± 1.3 *8.1*
Condition index	2.44 ± 0.9 *2.42*	1.94 ±0.32 *1.88*	1.92 ± 0.32 *1.92*	1.91 ±0.37 *1.87*
Relative organ size %				
Liver	3.3	2.7	2.8	2.8
Kidneys	1.4	1.4	1.4	1.4

Table 2 : body and organ weight (g), metals and total lipids concentrations as well as total loads, expressed as mean ± standard deviation and median (italic), for non cachectic (-), and cachectic guillemots, respectively +1 to +3 with increasing severity.

	Cachexia - n = 23	 +1 n = 31	 +2 n = 79	 +3 n = 29
Metals		***Liver***		
Zn µg/g dw	138.4 ± 27.3 *134.3*	144.6 ±40.1 *134.8*	159.5 ± 37.5 *153.0*	203.6 ± 64.4 *201.0*
Zn µg	899.3 ± 243 *911.4*	724.4 ± 260.0 *681.0*	721.4 ± 226.3 *693.2*	872.6 ± 470.3 *815.3*
Cu µg/g dw	48.9 ± 17.6 *44.9*	45.3 ± 15.4 *46.2*	59.1 ± 21.3 *59.7*	69.4 ± 32.0 *59.9*
Cu µg	302.5 ± 87.3 *268.3*	224.8 ± 79.2 *234.5*	259.6 ± 90.0 *260.5*	271.2 ± 92.5 *267.5*
Fe µg/g dw	2580 ± 1416 *2241*	2696 ± 1538 *2229*	3223 ± 1262 *3157*	4411 ± 2127 *4252*
Fe µg	16226 ± 6317 *16642*	12913 ± 7361 *11312*	14543 ± 6242 *14293*	17862 ± 8025 *16064*
Cd µg/g dw	1.9 ± 2.0 *1.4*	2.6 ± 1.7 *2.5*	2.7 ± 1.5 *2.6*	2.8 ± 1.9 *2.3*
Cd µg	11.8 ± 10.2 *8.5*	12.0 ± 7.3 *11.7*	11.7 ± 6.3 *11.0*	11.0 ± 7.0 *9.2*
Lipids g/g PS	0.18 ± 0.15 *0.14*	0.17 ± 0.07 *0.16*	0.16 ± 0.08 *0.15*	0.16 ± 0.05 *0.16*
Lipids g	1.22 ± 0.92 *0.90*	0.80 ± 0.40 *0.80*	0.74 ± 0.45 *0.63*	0.68 ± 0.35 *0.65*

	Cachexia			
	-	+1	+2	+3
	n = 23	n = 31	n = 79	n = 29
Metals				
		Kidneys		
Zn µg/g dw	157.6 ± 41.4 *155.0*	162.7 ± 31.3 *164.5*	179.1 ± 43.0 *181.1*	201.7 ± 37.5 *196.0*
Zn µg	350.6 ± 85.6 *342.2*	350.1 ± 64.1 *354.1*	347.9 ± 91.0 *336.6*	369.2 ± 75.7 *355.3*
Cu µg/g dw	21.7 ± 11.5 *21.0*	24.6 ± 8.7 *24.3*	33.7 ± 11.8 *32.7*	45.8 ± 17.0 *45.2*
Cu µg	45.1 ± 22.4 *44.7*	53.1 ± 17.2 56.6	64.9 ± 21.4 *63.4*	85.8 ± 40.8 *81.6*
Fe µg/g dw	744 ± 256 *725*	663 ± 260 *660*	655 ± 234 *627*	670 ± 224 *667*
Fe µg	1765 ± 792.1 *1768*	1520 ± 873 *1304*	1340 ± 695 *1151*	1267 ± 596 *1089*
Cd µg/g dw	7.1 ± 7.1 *5.2*	7.9 ± 5.0 *7.3*	8.7 ± 5.5 *7.9*	10.1 ± 6.0 *9.7*
Cd µg	15.5 ± 14.0 *10.3*	16.9 ± 11.3 *16.1*	16.9 ± 10.9 *15.7*	18.4 ± 11.2 *17.8*
Lipids g/g PS	n.d.	n.d.	n.d.	n.d.
Lipids g	n.d.	n.d.	n.d.	n.d.

Table 2 (continued): body and organ weight (g), metals and total lipids concentrations as well as total loads, expressed as mean ± standard deviation and median (italic), for non cachectic (-), and cachectic guillemots, respectively +1 to +3 with increasing severity. Not determined: n.d.; < dl: below detection limit.

	Cachexia			
	-	+1	+2	+3
	n = 23	n = 31	n = 79	n = 29
Metals				
		Muscle		
Zn µg/g dw	54.2 ± 9.4 *52.0*	63.9 ± 17.6 *60.0*	66.5 ± 15.8 *64.7*	79.3 ± 12.3 *77.4*
Zn µg	2070.0 ± 306.6 *2007.0*	2197.0 ± 565.7 *2020.0*	1987.9 ± 544.2 *19848.5*	1747.9 ± 254.9 *1739.0*
Cu µg/g dw	17.5 ± 4.2 *17.5*	19.5 ± 7.7 *17.6*	19.2 ± 6.0 *19.6*	19.8 ± 5.4 *19.8*
Cu µg	662.2 ± 111.0 *664.2*	662.9 ± 221.2 *626.5*	578.6 ± 181.2 *569.8*	442.3 ± 137.0 *431.8*
Fe µg/g dw	586 ± 107 *582*	689 ± 350 *592*	774 ± 280 *738*	1063 ± 384 *905*
Fe µg	22383 ± 3829 *21748*	23635 ± 10491 *20476*	23496 ± 8609 *22028*	23119 ± 6840 *22231*
Cd µg/g dw	< dl	< dl	< dl	< dl
Cd µg	< dl	< dl	< dl	< dl
Lipids g/g PS	0.11 ± 0.05 *0.10*	0.10 ± 0.07 *0.09*	0.08 ± 0.04 *0.07*	0.08 ± 0.04 *0.08*
Lipids g	4.40 ± 2.47 *3.80*	3.63 ± 2.94 *2.82*	2.42 ± 1.36 *2.13*	1.67 ± 0.81 *1.72*

The animal body weight reflects its cachectic status whatever the winter month considered: no statistically significant differences appear neither for cachectic or for non cachectic birds throughout the winter months (figure 2).

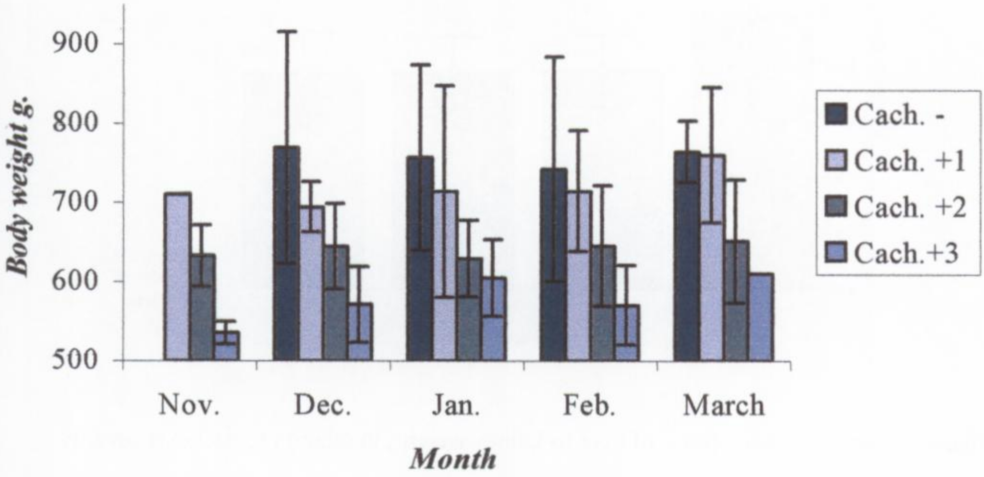


Figure 2: total body weight (g.) in relation to cachexia severity and winter month considered.

As one might expect, parallel to this body weight loss, the organs of these animals also exhibit a significantly decreased mass (liver, $r = -0.46$, $p< 0.01$; kidneys, $r = -0.38$, $p< 0.01$) in relation to the cachectic status. In particular, the liver weight loss is more pronounced than the kidney weight loss. A liver to kidney weight ratio was calculated and considered as a condition index for each bird (table 2), as proposed by Wenzel & Adelung (1996). A significant decrease of this condition index is noted with increased emaciation ($r = -0.29$, $p< 0.01$), with lowest condition index observed for highly cachectic birds (figure 3). In addition, results underline the absence of intestinal content as well as a slight dehydration of the tissues (1.6, 1.2 and 0.2 % water loss for liver, kidneys and pectoral muscle respectively), significantly correlated to the cachectic status, all with $p< 0.01$.

In most cases, among non emaciated guillemots, very little amount of subcutaneous and/or abdominal fat was noted during necropsy, except for four individuals which displayed substantial fat deposits and presented a higher mean body weight (923 g) compared to the rest of the sample. Three of these four animals were immature females collected early in December 1997, the sex of the fourth could not be determined. When treated separately and compared to the rest of the non cachectic birds, a general trend pointing towards lower metal levels in the tissues appeared. Unfortunately, due to a small sample size ($n = 4$), statistical comparisons demonstrating significant differences between both groups could not be made. Nevertheless, regarding their characteristics (higher mean body weight, presence of subcutaneous and abdominal fat, generalized lower metal levels) it is suggested that these four individuals represent a fitter class among the stranded non cachectic guillemots sampled and have thus been considered apart.

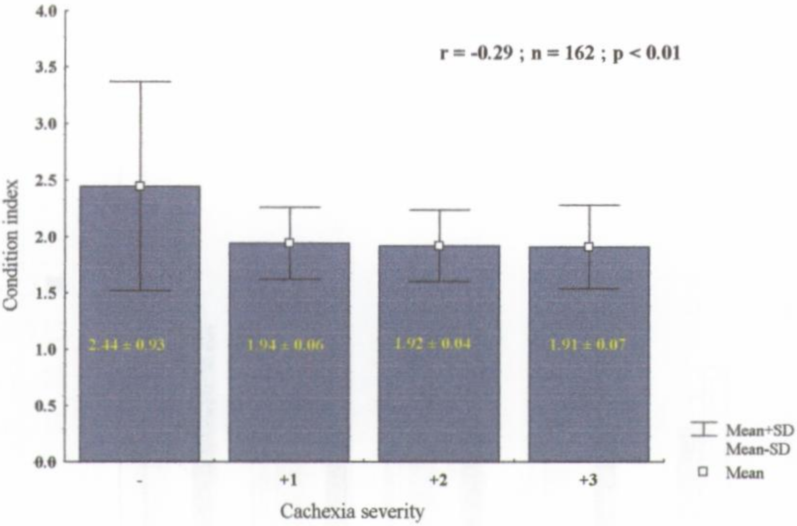


Figure 3: condition index (ratio of liver to kidney weight) in relation to cachexia severity.

Apart from cachexia, presence of GEAH is also largely noted, touching 75.3 % of the population (table 1). The frequency of this lesion is not statistically influenced by parameters like age, sex, wintering months considered, cachexia severity, presence or absence of oil.

Toxicology

External factors like short term fasting or starvation, through decomposition of energy storage and mobilization of its contents, are known to influence the metabolism of trace elements and in particular essential elements like Zn and Cu (Quaterman & Morrisson, 1981; Cousins, 1985; Krämer *et al.*, 1993; Cork *et al.*, 1994). In wild birds, this was described by Norheim & Borch-Iohnsen (1990) for the eider *Somateria mollissima*, by Wenzel & Adelung (1996) for the common guillemot and also by Esselink and co-workers (1995) for a land-based species, the barn owl (*Tyto alba guttata*). In a previous paper, Debacker *et al.* (1997) mentioned higher metal levels associated with a cachectic status although only presence or absence was considered. The results of the present study further confirm this result and, more important, clearly point towards a general rise of heavy metal levels in the organs as the cachectic status worsens: the more the guillemots lose weight and get severely emaciated, the higher the metal levels in the tissues (table 2). This obviously results from the loss of weight of the organs, but as additional parameters could contribute to explain the metal levels encountered, a statistical multiple regression analysis taking into account the age, sex, cachectic status, presence or not of oil and presence or not of GEAH was made. Cu and Zn concentrations in both liver and kidneys as well as Zn in muscles are highly dependent on the cachectic status (table 3). None of the pre-cited factors seems to influence Cu distribution in the muscles. On the other hand Cd concentrations in liver and kidneys are mainly dependent on the age of the individuals without any significant correlation with the cachectic status. Cd tends to accumulate in adult tissues compared to juveniles and immatures, as described in several others studies (Thompson, 1990; Lock *et al.*, 1992; Stewart *et al.*, 1994; Stewart, 1996; Wenzel & Adelung, 1996). This probably reflects the very long Cd biological half-life in biological tissues. Iron levels are strongly linked to cachexia in liver and muscles and to a lesser extent to oiling in liver and GEAH in muscles. In kidneys, Fe levels are linked to oiling alone. Total lipid content of the liver significantly decreases in birds fouled with oil, a probable consequence of an increased energy demand to face insulation loss. Lower hepatic total lipid concentrations are also found in younger birds. In muscles, the total lipid level is dependent on the cachectic status alone and follows a decreasing trend parallel to cachexia severity.

Variable	Age	Sex	Cachexia	Oiling	GEAH
Body weight	n.s.	n.s.	- - -	n.s.	n.s.
Liver weight	n.s.	-	- - -	n.s.	- -
Kidneys weight	n.s.	n.s.	- - -	n.s.	-
% H_2O liver	n.s.	n.s.	- -	n.s.	n.s.
%H_2O kidneys	n.s.	n.s.	- - -	n.s.	n.s.
% H_2O muscle	n.s.	n.s.	-	n.s.	n.s.
Condition index	n.s.	- -	- - -	n.s.	n.s.
Liver					
Cu	n.s.	n.s.	+ +	n.s.	n.s.
Zn	n.s.	n.s.	+ + +	n.s.	n.s.
Fe	n.s.	n.s.	+ + +	+	n.s.
Cd	+ + +	n.s.	n.s.	n.s.	n.s.
Total lipids	+	n.s.	n.s.	- - -	n.s.
Kidneys					
Cu	n.s.	n.s.	+ + +	n.s.	n.s.
Zn	n.s.	n.s.	+ + +	n.s.	+
Fe	n.s.	n.s.	n.s.	+ + +	n.s.
Cd	+ + +	n.s.	n.s.	n.s.	n.s.
Pectoral muscles					
Cu	n.s.	n.s.	n.s.	n.s.	n.s.
Zn	n.s.	n.s.	+ + +	n.s.	n.s.
Fe	n.s.	n.s.	+ + +	n.s.	-
Total lipids	n.s.	n.s.	- - -	n.s.	n.s.

Table 3: influence of different parameters on body condition and metal concentrations in the tissues. The symbol '+' or '-' indicates an increasing or decreasing trend and the amount of the symbols indicates their statistical relevance: + or - = significant at $p < 0.05$; ++ or - - = $p < 0.01$; +++ or - - - = $p < 0.001$; n.s. = not significant.

Compared to concentrations, the cachectic status of the animal is of little influence on the organs' total metal loads (table 2) except in the case of Cu in both kidneys and pectoral muscle. Total lipids burdens in liver and pectoral muscle also decrease sharply ($p< 0.001$ and $p< 0.0001$, respectively) which further confirms the complete use of fat reserves as energy source prior to protein catabolism. As expected, the Cd total burden increases significantly with the age of the bird. Oiling is of major importance on liver and kidneys Fe total loads (fig. 4) with significantly higher loads in oiled individuals. This could be due to destruction of the red blood cells following oil ingestion, with consecutive Fe release; a situation described for the Atlantic puffin, *Fratercula arctica* and herring gull, *Larus argentatus* (Leighton *et al*., 1983) but also for the common guillemot (Fry & Lowenstine, 1985; Khan & Ryan, 1991). However, in the case of stranded birds, post mortem oiling of the carcass cannot be ruled out.

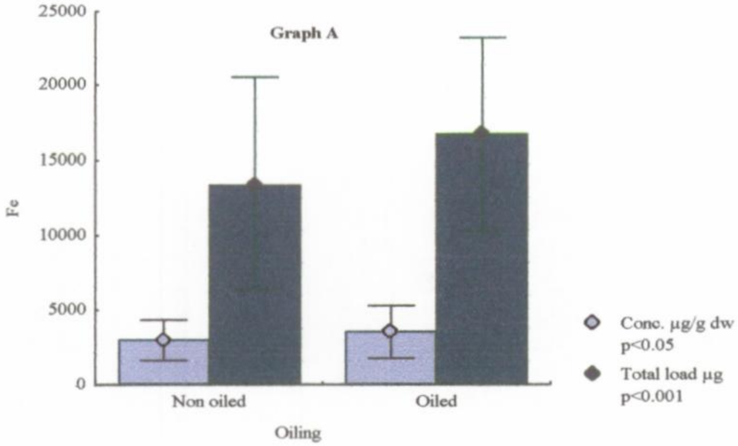
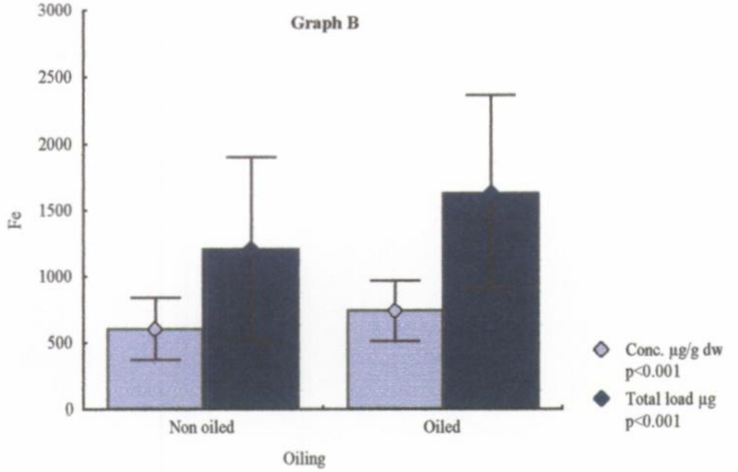


Figure 4: hepatic (graph A) and renal (graph B) iron concentration (µg g^{-1} dw) and total load (µg) of common guillemots.



Conclusions

Heavy metals absorption depends on a variety of factors often directly related to the metabolism and physiology of the animal. Metabolic adaptation and responses to stressors are more efficient for healthy birds compared to birds in poor physical conditions. This may reflect the ability of healthy birds to mobilize fat as primary energetic source and spare their protein reserves while birds in poor body condition depend primarily on protein catabolism for energy in emergencies (Heath & Dufty, 1998). In view of this, cachexia linked to starvation clearly influenced heavy metals levels, especially Cu and Zn, in the tissues. In addition, little modifications of the organ's total metal burdens parallel to cachexia severity further indicated a general re-distribution of heavy metals within the organs as a result of protein catabolism. Excesses of Cu and Zn, although essential to life, could be particularly toxic to the guillemots and could well represent an additional source of stress to birds already facing stressful conditions. Study of metal speciation in relation to the individual body condition should provide further information on the potentially toxic effects of these high metal levels.

Acknowledgements

This study was financed by the 'Impulse Programme in Marine Sciences supported by the Belgian state - The Prime Minister's Services - Scientific, Technical and Cultural Affairs.' (MS/12/032, MS/12/033, MN/DD/50 and MN/DD/51). The authors wish to thank P. Meire, H. Offringa, J. Seys, J. Van Waeyenberg, W. Roggeman and J. Tavernier for collecting and providing the specimens. Special thanks to R. Biondo for his valuable technical assistance and L. Seidel for her help with the statistical analysis.

References

BARNES, H. & BLACKSTOCK, J. (1973). Estimation of lipids in marine animals and tissues: detailed investigation of the sulphosphovanillin method for 'total' lipids. Journal of Experimental marine Biology and Ecology 12: 103-118.

BARRETT, R.T., PETERZ, M., FURNESS, R.W., DURINCK, J. (1989). The variability of biometric measurements. Ringing and Migration 10: 13-16.

BÉDART, J. (1985). Evolution and characteristics of the Atlantic Alcidae. In: Nettleship, D.N. & Birkhead, T.R. (Eds). The Atlantic Alcidae: the evolution, distribution and biology of the Auks inhabiting the Atlantic Ocean and adjacent water areas. Academic Press, London, 1-50.

CAMPHUYSEN, C.J. (1989). Beached Bird Surveys in The Netherlands 1915/1988. Seabird mortality in the Southern North Sea since the early days of oil pollution. Werkgroep Noordzee, Amsterdam.

CAMPHUYSEN C.J. & VAN FRANEKER J.A. (1992). The value of beached bird surveys in monitoring marine oil pollution. Techn. Rapp. Vogelbescherming 10. Vogelbescherming Nederland, Zeist, 191 pp.

CORK, S.C., ALLEY, M.R., STOCKDALE, P.H.G. (1994). A quantitative assessment of haemosiderosis in wild and captive birds using image analysis. Avian Pathology 24: 239-254.

COULSON, J.C., MONAGHAN, P., BUTTERFIELD, J., DUNCAN, N., THOMAS, C., SHEDDEN, C. (1983). Seasonal changes in the Herring Gull in Britain: weight, moult and mortality. Ardea 71: 235-244.

COUSINS, R.J. (1985). Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiological Reviews 65 (2): 238-308.

DEBACKER, V., HOLSBEEK, L., TAPIA, G., GOBERT, S., JOIRIS, C.R., JAUNIAUX, T., COIGNOUL, F., BOUQUEGNEAU, J.-M. (1997). Ecotoxicological and pathological studies of common guillemots *Uria aalge* beached on the Belgian coast during six successive wintering periods (1989-90 to 1994-95). Diseases of Aquatic Organisms 29: 159-168.

ESSELINK, H., VAN DER GELD, F.M., JAGER, L.P., POSTHUMA-TRUMPIE, G.A., ZOUN, P.E.F., BAARS, A.J. (1995). Biomonitoring heavy metals using the Barn Owl (*Tyto alba guttata*): sources of variation especially relating to body condition. Archives of Environmental Contamination and Toxicology 28: 471-486.

FOLCH, J., LEES, M., SLOANE STANLEY, G.H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biological Chemistry 226: 497-509.

FRY, D.M. & LOWENSTINE, L.J. (1985). Pathology of common murres and Cassin's auklets exposed to oil. Archives of Environmental Contamination and Toxicology 14 : 725-737.

FURNESS, R.W., THOMPSON, D.R., HARRISSON, N. (1994). Biometrics and seasonal changes in body composition of Common Guillemots *Uria aalge* from north-west Scotland. Seabird 16: 22-29.

HARRIS, M.P. & WANLESS, S. (1988). Measurements and seasonal changes in weight of Guillemots *Uria aalge* at a breeding colony. Ringing and Migration. 9: 32-36.

HEATH, J.A. & DUFTY, A.M. Jr. (1998). Body condition and the adrenal response in captive American kestrel juveniles. Physiological Zoology 71 (1): 67-73.

HERZBERG, G.R. (1991). Blood amino acids and the distribution of alanine and aspartate amino transferases in fed and fasted murres (*Uria aalge*). Comparative Biochemistry and Physiology 98A (3-4): 563-566.

HOPE JONES, P., BARRETT, C.F., MUDGE, G.P., HARRIS, M.P. (1984). Physical condition of auks beached in eastern Britain during the wreck of February 1983. Bird Study 31: 95-98.

HUETHER, S.E. & MCCANCE, K.L. (1994). Alterations of digestive functions. In: McCance, K.L. & Huether, S.E. (Eds). Pathophysiology: the biologic basis for disease in adults and children. St Louis, USA, 1320-1401.

JAUNIAUX, T., BROSENS, L., FARNIR, F., MANTECA, C., LOSSON, B., TAVERNIER J., VINDEVOGEL, H., COIGNOUL, F. (1996). Mortalité des oiseaux marins lors de l'hiver 1992-1993 le long du littoral belge. Annales de Médecine Vétérinaire 140: 149-159.

JAUNIAUX, T., BROSENS, L., MEIRE, P., OFFRINGA, H., COIGNOUL, F. (1998). Pathological investigations on guillemots (*Uria aalge*) stranded at the Belgian coast during the winter 1993-94. Veterinary Record 143: 387-390.

JOIRIS, C. R., TAPIA, G., HOLSBEEK, L. (1997). Increase of organochlorines and mercury levels in common guillemots *Uria aalge* during winter in the Southern North Sea. Marine Pollution Bulletin 34: 1049-1057.

KHAN, R.A. & RYAN, P. (1991). Long term effects of crude oil on common murres (*Uria aalge*) following rehabilitation. Bulletin of Environmental Contamination and Toxicology 46: 216-222.

KRÄMER, K., MARKWITAN, A., MENNE, A., PALLAUF, J. (1993). Zinc metabolism in fasted rats. Journal of Trace Elements and Electrolytes in Health and Disease 7: 141-146.

LEIGHTON, F.A., PEAKALL, D.B., BUTLER, R.G. (1983). Heinz-body hemolytic anemia from the ingestion of crude oil: a primary toxic effect in marine birds. Science 220: 871-873.

LOCK, J.W., THOMPSON, D.R., FURNESS, R.W., BARTLE, J.A. (1992). Metal concentrations in seabirds of the New Zealand region. Environmental Pollution 75: 289-300.

NORHEIM, G. & BORCH-IOHNSEN, B. (1990). Chemical and morphological studies of liver from eider (*Somateria mollissima*) in Svalbard with special reference to the distribution of copper. Journal of Comparative Pathology 102 : 457- 466.

OSBORN, D. & HARRIS, M.P. (1984). Organ weights and body composition in three seabirds species. Ornis Scandinavia 15: 95-97.

OSBORN, D., YOUNG, W.J., GORE, D.J. (1984). Pollutants in auks from the 1983 North Sea bird wreck. Bird Study 31: 99-102.

PIATT, J.F. & VAN PELT, T.I. (1997). Mass-mortality of Guillemots (*Uria aalge*) in the Gulf of Alaska in 1993. Marine Pollution Bulletin 34 (8):656-662.

QUATERMAN, J. & MORRISSON, E. (1981). The effect of short periods of fasting on the absorption of heavy metals. British Journal of Nutrition 46: 277-287.

QUEVAUVILLER, P. (1997). The QUASIMEME Project from a European Union Viewpoint Marine Pollution Bulletin 35 (1-6): 140-145.

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.

STEWART, F.M. (1996). Heavy metals and metallothioneins in seabirds, with special reference to their use as biomonitors. Ph.D. Thesis, Faculty of Science, University of Glasgow, 185 pp.

TAPIA, G.R. (1998). Mercury and organochlorines in the common guillemot *Uria aalge* wintering in the southern North Sea. Ph.D Thesis, Faculty of Medecine and Pharmacy, Free University of Brussels, Belgium.

THOMPSON D.R. (1990). Metal levels in marine vertebrates. In: Furness, R.W. & Rainbow, P.S. (Eds). Heavy metals in the marine environment. CRC Press, London, 143-181.

WENZEL, C. & ADELUNG, D. (1996). The suitability of oiled Guillemots (*Uria aalge*) as monitoring organisms for geographical comparisons of trace element contaminants. Archives of Environmental Contamination and Toxicology 31: 368-377.

Chapter 4

Body condition and heavy metals : comparison between guillemots (*Uria aalge*) found stranded at the Belgian coast and those caught in the Erika's oil spill (Brittany, December 1999).

To be submitted.

Contents

Body condition and heavy metals : comparison between guillemots (*Uria aalge*) found stranded at the Belgian coast and those caught in the Erika's oil spill (Brittany, December 1999).

Introduction

On 12 December 1999 the Erika broke in two off the coast of Brittany, France, whilst carrying approximately 30 000 tonnes of heavy fuel oil. Some 14 000 tons were spilled, and an estimated 14 600 tons remained in the sunken parts. The released oil rapidly washed ashore and spoiled about 400 km of coastline, killing thousands of seabirds. Among those, the common guillemot *Uria aalge* outnumbers by far all other species, representing more than 80 % of the total casualties (LPO, June 2000).

Following close contacts with the French parties, the Belgian MARIN group was asked to carry out necropsies and sampling on approximately 100 heavily oiled seabirds, mainly common guillemots.

Although oil had undoubtedly a direct mortality effect in this case, this sample was nevertheless interesting on a toxicological basis for further comparison with the toxicological results obtained on debilitated guillemots found stranded at the Belgian coast. Indeed, compared to stranded specimens, healthy and robust individuals off the Brittany coasts were also caught in this major oiling pollution, offering a possibility to examine heavy metal levels in more robust specimens. In addition, comparing guillemots from two distinct areas offered a possibility to investigate a possible geographical variation of the heavy metal levels encountered.

Material and methods

Collection and storage

A total of 72 guillemots collected on the Finistère coasts (Brittany, France) were made available for toxicological investigations. All were found dead on the beaches. All of them presented oil residues on the plumage and none had been cleaned prior to collection. Fresh carcasses were necropsied according to a standardized protocol (Jauniaux *et al.*, 1996), while putrefied specimens were discarded. Two age categories were considered as the individuals were aged as juveniles (<1 year old) or adult (potential but not necessarily breeders) based on the presence of the cloacal bursa (Camphuysen & van Franeker, 1992). Sex, total body weight, liver and kidney weight, as well as the presence of oil, intestinal contents, **a**cute **h**emorrhagic **g**astro-**e**nteropathy - GEAH - and other lesions were recorded. An exact determination of the age and/or sex could not be made for some individuals due to partial degradation of the tissues. Emaciation (cachexia) was evaluated using visible signs - presence or absence of subcutaneous fat, light to severe atrophy of the pectoral muscles - and given a range from 1 to 3, depending on its severity: specifically, 1: absence of fat and slight pectoral muscle atrophy; 2: moderate pectoral muscle atrophy; 3: severe pectoral muscle atrophy. Organs were collected (typically liver, kidney and pectoral muscle), weighed and kept frozen (-18° C) prior to toxicological analyses. Sub-cutaneous and abdominal fat were also sampled when possible. A condition index was calculated using the liver to kidney weight ratio, as proposed by Wenzel & Adelung (1996). This condition index is also significantly negatively correlated to the cachectic status of the bird: the lower the condition index, the more cachectic the bird (chapter 3). Guillemots with a condition index $\leqslant 2$ were considered as severely emaciated, those with a condition index ranging from 2.01 to 2.50 as moderately emaciated, and those displaying a condition index > 2.5 as robust specimens.

Heavy metals analysis

Atomic absorption spectrophotometry (ARL 3510) was used to determine heavy metals concentrations (Cu, Zn, Cd, Fe). Pb, Ni, Cr and Ti contents were also determined but the results were most often below the detection limits and will not be discussed here. After being weighed and dried during 48 hours at 110°C, samples were digested using a mixed solution of chloric and nitric acids (1:3, v:v) and slowly heated to 100°C until complete digestion. The samples were then diluted, filtered and analysed. In addition, quality of the analyses was controlled through participation to an intercalibration program (Quevauviller, 1997). Recoveries ranged from 97 to 100 % for Cu and Zn respectively, and 102 % for Cd. A set of certified material (DORM-2, National Research Council, Canada) gave a recovery rate of 92 % for Fe. Limits of detection were 0.18, 0.17, 0.16 and 0.18 $\mu g\ g^{-1}$ dw for Cu, Zn, Fe and Cd, respectively.

Statistical analysis

Statistical analysis of the data was performed using SAS statistical package (SAS Institute, Cary, NC, USA, version 6.12, 1996). Kolmogorv-Smirnov tests were used to check if the variables fitted a normal distribution. When not distributed normally the variables were log-transformed to normalize their distribution. One-way Anova tests were applied to test for significant differences among the different groups. Results were considered to be significant at the 5 % critical level ($p < 0.05$) and highly significant at the 1 % critical level ($p < 0.01$).

Results and discussion

General characteristics

A majority of the birds analyzed were juvenile guillemots, and more specifically juvenile female (44.4 %) and male (26.4 %) birds. Almost all were heavily oiled with oil residues covering 75 to 100 % of their body surface while only a few specimens were lightly touched (table 1). Visible signs of emaciation (cachectic status) were recorded in 58.3 % of the cases, which is a lower proportion than reported for guillemots collected on Belgian beaches (85.8 %). Similarly, when evaluating their body condition using the condition index (liver to kidney mass ratio), results show that a higher proportion of Brittany guillemots displayed a better condition index (> 2.5) (figure 1). In addition, the remaining non cachectic specimens (41.7 %) also appeared to be in a relatively better health status as subcutaneous and/or abdominal fat could be sampled for half of them. In those particular individuals, the condition index also tends to increase compared to non cachectic individuals, which did not present any fat deposits (respectively 2.7 ± 0.4 and 2.2 ± 0.6).

	Age			Sex			Cachexia				GEAH			Oiling	
	Ad.	Juv.	nd	Male	Female	nd	-	+1	+2	+3	+	-	nd	⩽50 %	⩾75 %
n	17	52	3	22	47	3	30	16	10	16	36	33	3	8	64
%	23.6	72.2	4.1	30.6	65.3	4.1	41.7	22.2	13.9	22.2	50	45.8	4.1	11.1	88.9

Table 1 : sample size (n) and percentage of juvenile and adult, male and female, non cachectic (-) and cachectic with increasing severity (+1 to +3), acute hemorrhagic gastro-enteropathy

(GEAH) presence (+) or absence (-), and oiling (expressed in percent of oiled body surface) of common guillemots collected on Brittany's beaches. Non determined: nd.

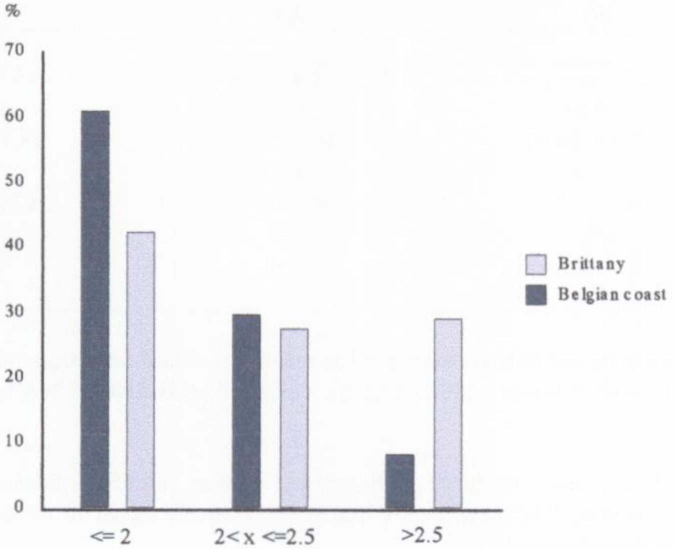


Figure 1 : comparison between guillemots of both geographical origin based on their condition index distribution.

Toxicological results

A general increase of the heavy metal levels is observed with decreasing body condition (decreased condition index, increasing cachectic status), a likely effect of a general redistribution of the heavy metals within the organs during starvation (table 2). These results agree with results previously described for guillemots collected at the Belgian coast (chapter 3).

	Condition index		
	⩽2 n = 29	2 <x ⩽ 2.5 n = 19	> 2.5 n = 24
Liver			
Cu	66.9 ± 25.7	34.1 ± 9.0	23.7 ± 4.4
	67.9	33.1	25.1
Zn	147.5 ± 46.8	118.2 ± 23.2	94.6 ± 14.6
	137.9	115.3	93.1
Fe	3866 ± 1201	2582 ± 1022	1802 ± 643
	3764	2521	1682
Cd	4.4 ± 3.0	2.0 ± 2.2	1.0 ± 0.7
	3.7	1.2	0.9
Kidneys			
Cu	23.1 ± 10.2	14.2 ± 2.8	12.8 ± 1.0
	22.3	13.7	12.6
Zn	160.3 ± 41.7	126.6 ± 23.4	113.9 ± 16.7
	158.9	120.0	112.5

Fe	770 ± 240 729	839 ± 43 829	831 ± 137 815
Cd	13.2 ± 12.1 9.1	7.8 ± 8.2 3.6	5.4 ± 5.4 3.4
Muscle			
Cu	17.9 ± 4.1 16.9	14.4 ± 2.5 13.8	15.5 ± 4.0 15.2
Zn	57.3 ± 14.9 57.8	46.5 ± 7.1 46.3	48.5 ± 18.7 43.1
Fe	687 ± 177 686	509 ± 77 526	523 ± 180 464
Cd	< dl	< dl	< dl

Table 2 : trace elements concentrations expressed as mean ± standard deviation and median (µg/g dw) in guillemots of different condition index, collected on Brittany's beaches. < dl : below detection limit.

When comparing heavy metal levels in guillemots of similar condition index from Brittany and Belgian coasts it appears that significantly higher levels are found in all three tissues of the Belgian guillemots (tables 3, 4 and 5).

Condition index ⩽ 2					
Tissue	Brittany		Belgian	F	p
Liver					
Cu	66.9 ± 25.7		64.0 ± 24.2	$F_{1,125} = 0.31$	n.s.
Zn	147.5 ± 46.8		162.1 ± 43.2	$F_{1,125} = 2.41$	n.s.
Fe	3866 ± 1201		3348 ± 1749	$F_{1,125} = 2.16$	n.s.
Cd	4.38 ± 3.03	↘↘	2.94 ± 1.88	$F_{1,125} = 9.47$	**
Kidneys					
Cu	23.1 ± 10.2	↗↗↗↗	34.5 ± 12.9	$F_{1,125} = 19.15$	****
Zn	160.3 ± 41.7	↗↗	187.8 ± 39.6	$F_{1,125} = 10.56$	**
Fe	770 ± 240	↗	645 ± 232	$F_{1,125} = 6.43$	*
Cd	13.24 ± 12.10	↘	9.62 ± 6.15	$F_{1,125} = 4.72$	*
Muscle					
Cu	17.9 ± 4.1		19.7 ± 6.6	$F_{1,127} = 1.90$	n.s.
Zn	57.3 ± 14.9	↗↗↗	69.0 ± 16.7	$F_{1,127} = 11.52$	***
Fe	687 ± 177		796 ± 343	$F_{1,127} = 2.74$	n.s.
Cd	< dl		< dl		

Table 3 : trace elements concentrations expressed as mean ± standard deviation (µg/g dw) in severely emaciated guillemots collected on the Belgian and Brittany coasts. < dl : below detection limit. Arrows indicate an increasing ↗ or decreasing ↘ trend (* : $p<0.05$; ** : $p<0.01$; *** : $p< 0.001$; **** : $p<0.0001$).

In those severely emaciated individuals (condition index ⩽ 2, table 3), while hepatic Cu and Zn levels remain similar between the two groups groups, renal concentrations significantly increase for Belgian specimens by a factor of, respectively, 1.5 and 1.2. On the contrary, hepatic and renal Cd concentrations are significantly higher in Brittany birds. Indeed, among those severely emaciated guillemots, a few adult females display particularly high Cd concentrations (up to 11 and 44 µg/g dw respectively in the liver and kidneys) compared to the mean hepatic and renal concentrations found in their group (respectively 4.4 and 13.2 µg/g dw). Guillemots wintering off the Brittany coasts mainly originate from the Irish and Celtic Sea and from southwest Scotland while individuals from the North Sea colonies are occasional visitors (Baille *et al.*, 1994 ; B. Cadiou, Bretagne Vivante-SEPNB, *pers. com.*). In these areas relatively low Cd levels are reported, especially in offshore areas (*e.g.* ± 0.1 ppm ww in dab , plaice and flounder liver - Marine Pollution Monitoring Management Group, 1998). However, local coastal sites like the Severn estuary, along which guillemots colonies are found, are particularly well known for their high Cd inputs from industrial and/or domestic sources (Noël-Lambot *et al.*, 1980 ; Ferns, 1984). Cd accumulates with age (Thompson, 1990 ; Lock *et al.*, 1992 ; Stewart *et al.*, 1994 ; Wenzel & Adelung, 1996) and potential contamination of these adult females while breeding in the vicinity of more polluted sites cannot be excluded.

			Condition index $2 < x \leqslant 2.5$		
Tissue	Brittany		Belgian	F	p
Liver					
Cu	34.1 ± 9.0	↗↗	48.3 ± 17.2	$F_{1,65} = 11.70$	**
Zn	118.2 ± 23.2	↗↗↗	165.7 ± 53.0	$F_{1,65} = 14.08$	***
Fe	2582 ± 1022		3273 ± 1393	$F_{1,65} = 3.85$	n.s.
Cd	1.95 ± 2.21		2.11 ± 1.07	$F_{1,65} = 0.15$	n.s.
Kidneys					
Cu	14.2 ± 2.8	↗↗↗↗	31.0 ± 15.7	$F_{1,65} = 21.16$	****
Zn	126.6 ± 23.4	↗↗↗	163.0 ± 42.5	$F_{1,65} = 12.34$	***
Fe	839 ± 188		710 ± 261	$F_{1,65} = 3.82$	n.s.
Cd	7.76 ± 8.24		6.93 ± 4.68	$F_{1,65} = 0.26$	n.s.
Muscle					
Cu	14.6 ± 2.5	↗↗	18.1 ± 5.0	$F_{1,65} = 8.97$	**
Zn	46.5 ± 7.1	↗↗↗↗	63.4 ± 14.6	$F_{1,65} = 23.16$	****
Fe	509 ± 77	↗↗↗	771 ± 271	$F_{1,65} = 16.98$	***
Cd	< dl		< dl		

Table 4 : trace elements concentrations expressed as mean ± standard deviation (µg/g dw) in moderately emaciated guillemots collected on the Belgian and Brittany coasts. < dl : below detection limit. Arrows indicate an increasing ↗ or decreasing ↘ trend (* : p<0.05 ; ** : p<0.01 ; *** : p< 0.001 ; **** : p<0.0001).

The same remark applies to the moderately emaciated guillemots (condition index between 2.01 and 2.5, table 4), among which were found a few individuals with higher hepatic and renal Cd concentrations. Similarly, Cu levels significantly increase by a factor 1.3, 1.4 and 2.2, respectively, in increasing order, in the pectoral muscle, liver and kidneys. Hepatic, renal and pectoral muscle Zn levels also significantly increase, although to a lesser extent.

	Condition index > 2.5				
Tissue	Brittany		Belgian	F	p
Liver					
Cu	23.2 ± 4.1	↗↗	33.7 ± 11.2	$F_{1,27} = 12.11$	**
Zn	93.5 ± 14.4	↗↗	144.3 ± 58.8	$F_{1,27} = 11.23$	**
Fe	1808 ± 663		2328 ± 782	$F_{1,27} = 3.76$	n.s.
Cd	1.02 ± 0.69		1.36 ± 0.86	$F_{1,27} = 1.35$	n.s.
Kidneys					
Cu	12.8 ± 1.1	↗↗	25.5 ± 18.9	$F_{1,30} = 8.77$	**
Zn	115.0 ± 16.4	↗↗↗	150.0 ± 31.8	$F_{1,30} = 16.63$	***
Fe	836 ± 139.5		741.2 ± 212.5	$F_{1,30} = 2.34$	n.s.
Cd	5.57 ± 5.47		6.41 ± 4.76	$F_{1,30} = 0.20$	n.s.
Muscle					
Cu	14.5 ± 2.4	↗↗	18.4 ± 5.5	$F_{1,31} = 8.07$	**
Zn	46.1 ± 17.0	↗	61.8 ± 18.0	$F_{1,31} = 6.37$	*
Fe	495 ± 177	↗	755 ± 444	$F_{1,31} = 5.56$	*
Cd	< dl		< dl		

Table 5 : trace elements concentrations expressed as mean ± standard deviation (µg/g dw) in guillemots displaying a higher condition index collected on the Belgian and Brittany coasts.
< dl : below detection limit. Arrows indicate an increasing ↗ or decreasing ↘ trend (* : $p<0.05$; ** : $p<0.01$; *** : $p< 0.001$; **** : $p<0.0001$).

Significantly higher hepatic and renal Cu and Zn levels are also found in Belgian guillemots compared to their Brittany counterparts, even when comparing both groups of more robust individuals (condition index above 2.5, table 5).

These results suggest that, in addition to the heavy metal redistribution likely to occur in the organs during starvation, guillemots have to face environmental trace elements concentrations which tend to be higher in the southern North Sea compared to Brittany.

Although a general decrease of heavy metal levels has been observed in the North Sea during the last decade (Scholten *et al.*, 1998) this area still receives contaminant inputs through several large estuaries leading to higher trace element concentrations than those detected in the open Atlantic waters (Kremling, 1985 ; North Sea Task Force, 1993 ; Dauby *et al.*, 1994). A higher dietary intake could well be an explanation for these higher metal levels observed in Belgian guillemots, as they probably forage in more contaminated areas than those foraging in the Atlantic open waters off the Brittany coasts. These observations agree with those presented by Wenzel & Adelung (1996) comparing guillemots collected stranded or moribund at the German Bight with individuals collected in northern Brittany (Channel area) (table 6). Following their results, the authors underline that trace elements levels tend to be higher in the German Bight than northern Brittany. It is also worth noting that concentrations reported for stranded German guillemots are close to those reported for Belgian individuals.

Tissue	Cu	Zn	Cd	Source
Liver	48.1 ± 27.9	124.7 ± 40.4	2.77 ± 2.76	this study
	36.4	116.3	1.73	
	32.9 ± 23.8	118.5 ± 39.6	2.70 ± 4.67	Wenzel & Adelung, 1996.
	22.7	105.4	1.13	
Kidneys	17.7 ± 8.4	137.9 ± 37.0	9.51 ± 10.00	this study
	13.8	126.6	4.94	
	26.5 ± 13.4	137.1 ± 61.1	10.20 ± 14.90	Wenzel & Adelung, 1996.
	21.6	122.9	5.18	
Muscle	16.3 ± 3.9	51.6 ± 15.5	< dl	this study
	15.5	46.8		
	16.5 ± 5.9	57.2 ± 17.4	0.14 ± 0.40	Wenzel & Adelung, 1996.
	15.0	51.5	0.03	

Table 6 : trace elements concentrations expressed as mean ± standard deviation and median (µg/g dw) in guillemots collected in northern Brittany (English Channel area) by Wenzel & Adelung (1996) and those collected in the Finistère area (Atlantic side) for this study. < dl : below detection limit.

Conclusions

To conclude, results show that for a similar nutritional status, heavy metal concentrations and in particular hepatic and renal Cu levels tend to increase significantly in guillemots collected stranded at the Belgian coast. This is still true for the most robust individuals of both samples. This suggests that guillemots collected at the Belgian coast are foraging in a more contaminated area compared to their Brittany wintering counterparts. In addition to these higher local heavy metal levels, a complete redistribution of the contaminants occurs during the critical starving period when the animal is most susceptible to any additional external stressor. To what extent this trace elements redistribution in the organs acts as an additional stress remains to be determined through the study of Cu and Zn speciation in relation to the animal's general body condition.

Acknowledgements

This study was financed by the 'Impulse Programme in Marine Sciences supported by the Belgian state - The Prime Minister's Services - Scientific, Technical and Cultural Affairs. (MN/DD/50 and MN/DD/51). The authors wish to thank B. Cadiou (Bretagne Vivante – SEPNB) for providing the birds and the facilities, the veterinary volunteers Dr. J.-L. Castaigne and Dr. A. Joris for their assistance to carry out the necropsies, but also R. Biondo for his valuable technical assistance.

References

BAILLIE, S.R., DUDLEY, C., HARRIS, M.P. (1994). Atlas of recoveries of guillemots *Uria aalge* ringed in Scotland. Final Report to Scottish Natural Heritage.

CAMPHUYSEN C.J. & VAN FRANEKER J.A. (1992). The value of beached bird surveys in monitoring marine oil pollution. Techn. Rapp. Vogelbescherming 10. Vogelbescherming Nederland, Zeist, 191 pp.

DAUBY, P., FRANKIGNOULLE, M., GOBERT, S., BOUQUEGNEAU, J.-M. (1994). Distribution of POC, PON, and particulate Al, Cd, Cr, Cu, Pb, Ti, Zn and $\delta^{13}C$ in the English Channel and adjacent areas. Oceanologica Acta 17 (6): 643-657.

FERNS, P.N. (1984). Birds of the Bristol Channel and Severn estuary. Marine Pollution Bulletin 15 (2): 76-81.

JAUNIAUX, T., BROSENS, L., FARNIR, F., MANTECA, C., LOSSON, B., TAVERNIER J., VINDEVOGEL, H., COIGNOUL, F. (1996). Mortalité des oiseaux marins lors de l'hiver 1992-1993 le long du littoral belge. Annales de Médecine Vétérinaire 140: 149-159.

KREMLING, K. (1985). The distribution of copper, nickel, manganese, and aluminium in surface waters of the open Atlantic and European shelf area. Deep-Sea Research 32 (5): 531-555.

LOCK, J.W., THOMPSON, D.R., FURNESS, R.W., BARTLE, J.A. (1992). Metal concentrations in seabirds of the New Zealand region. Environmental Pollution 75: 289-300.

LPO, June 2000 : Ligue pour la Protection des Oiseaux. Official Website : http://assoc.wanadoo.fr/lpo-paris/.

MARINE POLLUTION MONITORING MANAGEMENT GROUP (1998). National Monitoring Program. Survey of the quality of UK coastal waters. Aberdeen, 79 pp.

NOËL-LAMBOT, F., J.-M. BOUQUEGNEAU, FRANKENNE, F., DISTECHE, A. (1980). Cadmium, zinc and copper accumulation in limpets (*Patella vulgata*) from the Bristol Channel with special reference to metallothioneins. Marine Ecology Progress Series 2 : 81-89.

NORTH SEA TASK FORCE REPORT (1993). North Sea quality status report. Oslo and Paris Commission (Olsen & Olsen, eds), 132 pp.

QUEVAUVILLER, P. (1997). The QUASIMEME Project from a European Union Viewpoint. Marine Pollution Bulletin 35 (1-6): 140-145.

SCHOLTEN, M.C.TH., KRAMER, K.J.M., LAANE, R.W.P.M. (1998). Trends and variation in concentration of dissolved metals (Cd, Cu, Pb, and Zn) in the North Sea (1980-1989). ICES Journal of Marine Science 55 : 825-834.

STEWART, F.M., THOMPSON, D.R., FURNESS, R.W., HARRISON, N. (1994). Seasonnal variation in heavy metal levels in tissues of common Guillemots, *Uria aalge* from Northwest Scotland. Archives of Environmental Contamination and Toxicology 27:168-175.

THOMPSON D.R. (1990). Metal levels in marine vertebrates. In: Furness, R.W. & Rainbow P.S. (Eds). Heavy metals in the marine environment. CRC Press, London, 143-181.

WENZEL, C. & ADELUNG, D. (1996). The suitability of oiled guillemots (*Uria aalge*) as monitoring organisms for geographical comparisons of trace element contaminants. Archives of Environmental Contamination and Toxicology 31 : 368-377.

Chapter 5

Combined effects of experimental heavy metals contamination (Cu, Zn and CH_3Hg) and starvation on the quails' body condition: parallelism with a wild common guillemots (*Uria aalge*) population found stranded at the Belgian coast.

Based on the manuscript by: V. Debacker[1], A. Rutten[1], T. Jauniaux[2], C. Daemers[1] and J.-M. Bouquegneau[1].

[1] Oceanology, University of Liège, B6 Sart Tilman, B-4000, Belgium.

[2] Pathology Department of the Veterinary College, University of Liège, B43 Sart Tilman, B-4000, Belgium.

Submitted to Biological Trace Element Research.

Contents

Combined effects of experimental heavy metals contamination (Cu, Zn and CH_3Hg) and starvation on the quails' body condition: parallelism with a wild common guillemots (*Uria aalge*) population found stranded at the Belgian coast.

Abstract

Combined effects of heavy metals contamination (Cu, Zn and CH_3Hg) and starvation were tested on common quails (*Coturnix coturnix*) and used as a model for comparison with a wild common guillemots (*Uria aalge*) population found stranded at the Belgian coast. Appropriate heavy metal levels were given to the quails to obtain concentrations similar to those found in the seabirds' tissues. The contaminated animals were then starved for 4 days to simulate the evident malnutrition symptoms observed at the guillemots' level. In such conditions, food intake and total body weight are shown to decrease in contaminated individuals with simultaneous significant hepatic and renal increase of the heavy metal concentrations. Alike in guillemots, higher heavy metal levels were observed in those contaminated quails which had also developed a cachectic status characterized by a general atrophy of their pectoral muscles and complete absence of subcutaneous and/or abdominal fat deposits. Although likely the result of a general protein catabolism during starvation, it is suggested that these higher metal levels could in turn favour a general lessened body condition (cachectic status).

Introduction

Common guillemots (*Uria aalge*) stranded along the Belgian coast have been studied for several years in the framework of a multidisciplinary approach involving both pathological and toxicological investigations. In this context, a clear relationship appeared between the individuals' body condition, and more specifically its emaciation status, and both the heavy metals distribution and their concentrations in the target organs (namely the liver and kidneys). In particular, in those organs sampled on emaciated guillemots, significantly higher Cu and Zn concentrations were detected and shown to significantly increase with increasing emaciation (Debacker *et al*., 1997, 2000 *in press*) while Hg and organochlorine levels were clearly shown to increase during wintering in the southern North Sea (Joiris *et al*., 1997; Tapia, 1998). In addition, when compared to more robust guillemots collected at colonies in more preserved areas of the North (Stewart *et al*., 1994; Wenzel & Gabrielsen, 1995), stranded guillemots at the Belgian coast displayed higher hepatic, renal and pectoral muscle levels of Cu, Zn and Hg. Such findings inevitably arises the question to know whether or not high heavy metal levels could induce emaciation, or, on the contrary, could these higher levels be the result of the emaciation process. To answer this, two set of experiments, using the common quail, *Coturnix coturnix*, as an experimental model, were carried out.

The first objective was to determine which heavy metal concentrations in the food could lead to similar levels in the organs as those observed in a wild population of common guillemots. Once determined, these particular heavy metal concentrations were used during the second experiment to contaminate a group of quails which were then starved during four days. Testing the impact of a starvation period on the heavy metal levels could then be assessed.

Materials and methods

Animals.

Seven weeks old male quails (*Coturnix coturnix*) were purchased from a breeding farm and randomly allocated to one of the following groups after one week acclimatisation. All quails were housed in a room equipped with a 12h light – 12 hour dark cycle, at room temperature (18-20°C), in individual cages (24 x 41 cm) with *ad libitum* access to tap water. The animals were fed (50g per day) with a special diet (commercially prepared) the principal components of which were fish and manioc flours (respectively 30 and 62 %), supplemented with vitamins (3 %) and antibiotics. Every day, prior to feeding, all individuals were weighed to the nearest 0.01 g (Sartorius 3713 MP) as well as the remaining food in the manger in order to determine, respectively, the gain or loss of weight and the quantity of food ingested. During the experiments, birds were regularly examined by veterinarians. Experiments and euthanasia protocols were submitted to the Veterinary Committee of the Belgian Ministry of Agriculture.

Food preparation.

Quails were fed contaminated food containing Cu, Zn and CH_3Hg (methylmercury) at different concentrations. Food was contaminated using solutions of copper chloride, zinc chloride and a standard solution of CH_3Hg. These solutions were sprayed upon the food (350 ml of solution/ 3.5 kg of food) which, at the same time, was homogenized using a rotating tank (capacity 3.5 kg). Food was then stored in polyethylene containers (capacity 500g) and kept refrigerated (2.5°C) prior to analyses. Uncontaminated food was sprayed upon with deionized water and stored in the same conditions. Samples of both uncontaminated and contaminated food of each food container used during the experiment, were analysed for their heavy metal contents prior to feeding the animals.

First experiment, part I: contamination with Cu and Zn using three different concentrations.

Control animals (n = 5) received uncontaminated food while contaminated food containing increasing metal concentrations were given to the three other groups (each with n = 5 individuals). Heavy metals concentrations in the food ranged from 1000 µg/g ww of Zn and 200 µg/g ww of Cu, 2000 µg/g ww of Zn and 400 µg/g ww of Cu, and, 3000 µg/g ww of Zn and 600 µg/g ww of Cu, respectively for group one (group CZ 1), two (group CZ 2) and three (group CZ 3). The experiment lasted for 15 days at the end of which all quails were euthanazied by decapitation and immediately necropsied and sampled. No quail died prior the end of the experiment.

First experiment, part II: contamination with CH_3Hg (2 µg/g ww).

In addition, the food was also contaminated with a solution of CH_3Hg (2 µg/g ww). Six quails were fed with this enriched methylmercury diet while the control group (n = 6) received an uncontaminated food. The experiment lasted for 15 days at the end of which all quails were euthanazied by decapitation and immediately necropsied and sampled. One contaminated quail died prior the end of the experiment. Results regarding this individual were discarded.

Second experiment: contamination with Cu, Zn and Hg at a given concentration, followed by a 4-day complete starvation.

During this second experiment, 12 control quails were fed uncontaminated food while 12 other individuals received a contaminated food containing 3000 µg/g ww of Zn, 600 µg/g ww of Cu and 2 µg/g ww of CH_3Hg. The contamination period lasted for 15 days at the end of which half the quails of each group were euthanazied by decapitation and immediately necropsied. The remaining control (n = 6) and contaminated (n = 6) individuals were then completely starved for four days and euthanazied in the same conditions at the end of this 96 hours period. No quail died prior the end of the experiment. Due to a reduced renal mass at the end of the starvation period, Hg analysis were performed in the liver and pectoral muscle of the control but non fasted group only. Hg levels were, however, below the detection limit for all control individuals.

Necropsies and sampling.

After two weeks of contamination, all individuals were killed by decapitation and immediately necropsied according to a standardized protocol (Jauniaux *et al.*, 1996). Body and organs weight (typically liver, kidneys and pectoral muscle) were recorded to the nearest 0.01 g (Sartorius 3713 MP). Pathological observations (presence or absence of **a**cute **h**emorrhagic **g**astro-**e**nteropathy – GEAH – possible congestion, parasites, …) were noted for each individual. For starved individuals, emaciation was evaluated and given a range from 0 to 3, depending on its severity (presence or absence of subcutaneous fat, light to severe atrophy of the pectoral muscles): specifically: 0: presence of subcutaneous fat, normal pectoral muscle; 1: absence of fat and slight pectoral muscle atrophy; 2: moderate pectoral muscle atrophy; 3: severe pectoral muscle atrophy. Liver, kidneys and part of the pectoral muscles were sampled for each animal and stored frozen (-18°C) in polyethylene flasks prior to analysis.

Heavy metals analysis.

Atomic absorption spectrophotometry (ARL 3510) was used to determine Cu and Zn concentrations. After being weighed and dried during 48 hours at 110°C, samples were digested using a mixed solution of chloric and nitric acids (1:3, v:v) and slowly heated to 100°C until complete digestion. Samples were then diluted using deionized water and filtered prior to analysis. In addition, the quality of the analyses was controlled through participation in an intercalibration program (Queveauviller, 1997). Recoveries ranged from 97 to 100 % for Cu and Zn respectively. Limits of detection were 0.18 and 0.17 $\mu g\ g^{-1}$ dw for Cu and Zn respectively. Given the heavy metal concentrations in each tissue (liver, kidneys and pectoral muscle) and their respective weight, heavy metal total loads were determined.

Total mercury analyses were performed by specific atomic absorption spectrometry using a Perkin-Elmer MAS-50 Mercury analyser after the method described by Hatch & Ott (1968), modified by Bouquegneau (1973). Limit of detection was 0.01 µg/g dw.

Data analysis.

All datas were treated using Statistica®5.1 for Windows. Mean values of parameters were tested for differences between contaminated and control groups using One-way ANOVA test followed by Tukey's post hoc tests. The results were considered as significant at the 5 % level ($p < 0.05$) and highly significant at the 1 % level ($p < 0.01$).

Results

First experiment, part I: contamination with Cu and Zn using three different concentrations.

During the experiment, control and contaminated CZ1 and CZ 2 groups ingested significantly more food (all $p < 0.01$) than the contaminated CZ 3 group. In this latter group, a significant decrease ($p < 0.05$) of their total body weight is observed after 15 days of contamination.

Compared to the control group, significantly increasing Cu and Zn concentrations are noted in both liver and kidneys of the contaminated groups. In the pectoral muscle, however, no significant trend is observed. The heavy metal levels detected in the contaminated quails's tissues were then compared to those observed in the tissues of wild common guillemots collected stranded at the Belgian coast (figure 1). The levels of the hepatic Cu and Zn concentrations noted for the guillemots were, respectively, between the levels observed for the contaminated groups of quails CZ 2 and CZ 3 and CZ 1 and CZ 2. In the kidneys, while Zn concentrations were slightly above those of the CZ 3 group, the Cu contents lie between the control and the CZ 1 groups. The guillemot's pectoral muscle Cu and Zn levels were always far above those of the CZ 3 contaminated group. In view of these comparisons, the experimental food contamination using 600 µg/g ww of Cu and 3000 µg/g ww of Zn applied to the CZ 3 group was selected to carry out the second experiment.

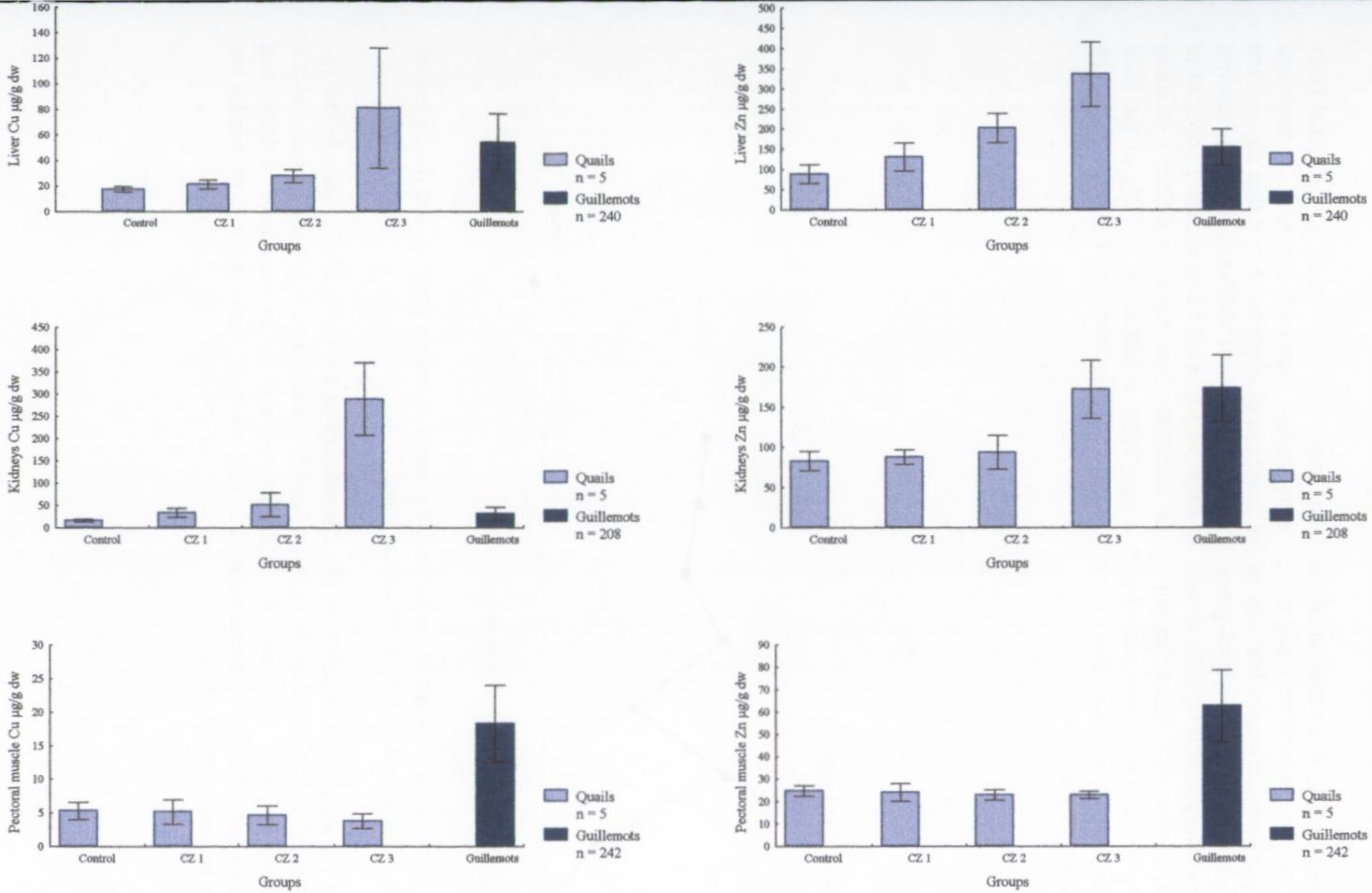


Figure 1: comparison of the Cu and Zn levels found in the guillemots' liver, kidneys and pectoral muscles, with those found in contaminated quails fed with increasing metal concentrations (CZ 1: 1000 µg/g dw of Zn, 200 µg/g dw of Cu; CZ 2: 2000 µg/g dw of Zn and 400 µg/g dw of Cu; CZ 3: 3000 µg/g dw of Zn and 600 µg/g dw of Cu).

First experiment, part II: contamination with CH_3Hg (2 µg/g ww).

During this experiment, the variation of the total body weight compared to the initial body weight (prior to the experiment) (figure 2) significantly increased for both control and contaminated quails until the very first day of the contamination period. From that day on, the total body weight of the contaminated group first decreased then stabilised while the control animals kept increasing their total body weight. In addition, while both contaminated and control groups ingested similar food quantities during the acclimatisation period, a different situation appeared during the contamination period with a significant reduction ($p < 0.05$) of the food intake of the contaminated group. The control group, on the contrary, kept ingesting as much food during the whole experiment.

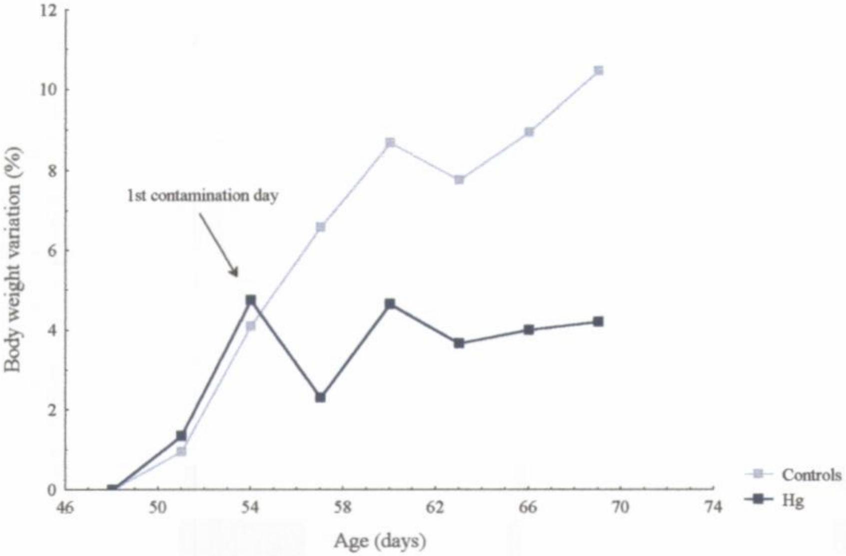


Figure 2: total body weight variations, expressed in percent, between controls and quails fed an enriched CH_3Hg diet, as a function of the animal's age.

At the end of the experiment, mean total Hg concentrations for control birds were below the detection limit while contaminated quails had significantly accumulated Hg in their tissues with a mean concentration of 14.3, 15.2 and 7.4 µg/g dw respectively in their liver, kidneys and pectoral muscles (figure 3). The concentration of 2 µg/g ww was been used to contaminate the food during the second experiment.

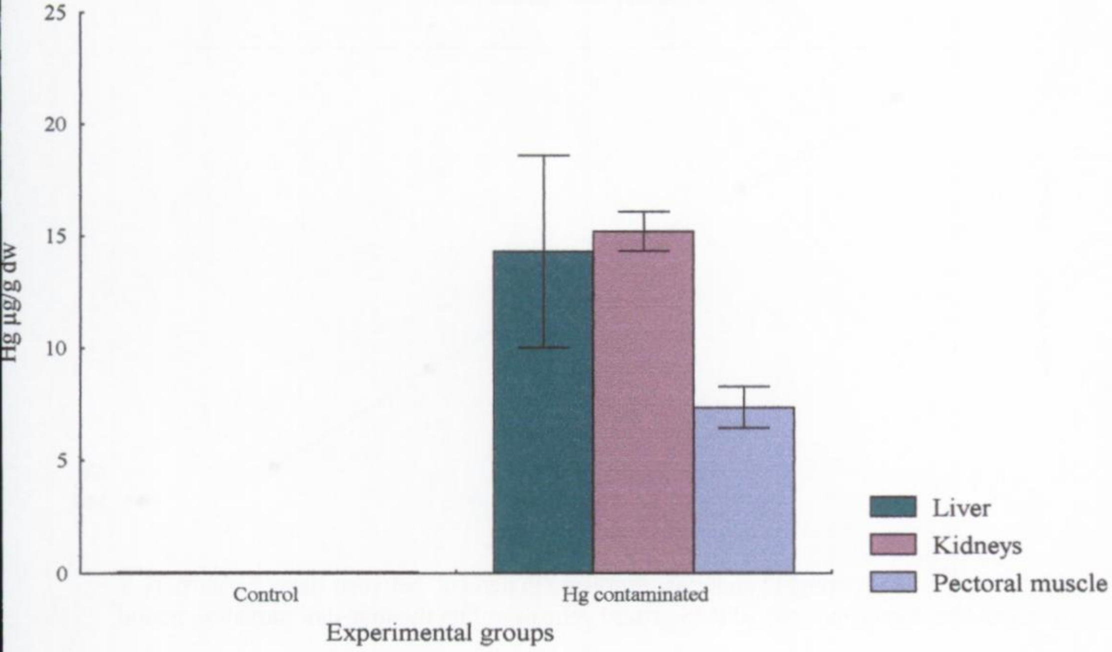


Figure 3: total Hg accumulation in the quail's tissues after two weeks of contamination

Second experiment: contamination with Cu, Zn and CH_3Hg at a given concentration, followed by a 4-day complete starvation.

Prior to the starvation period, the total body weight of the contaminated quails, although not significantly different from the control group, tends to be lower. Once starved, both control and contaminated individuals lost weight from day one to day four. However, for the contaminated group, while not significantly different, from day 2 to day 4, the body weight loss is more pronounced than for control animals, with respectively 29.8 % and 21.7 % (figure 4).

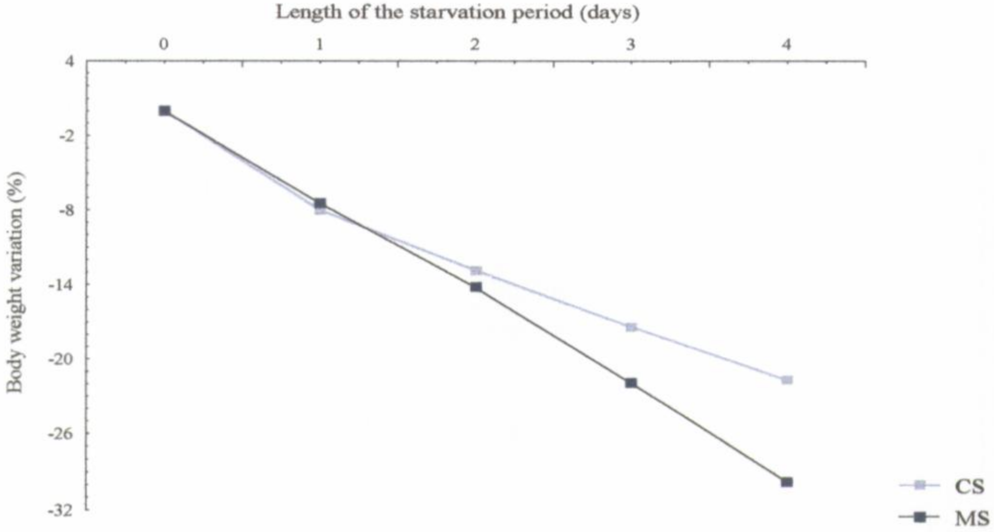


Figure 4: total body weight variations, expressed in percent, between starved control (CS group) and contaminated starved (MS group) animals during the four-day starvation period.

In addition, at the end of this 4 days starvation period, hepatic and renal weight losses were recorded for both starved groups. These losses were, however, more pronounced in the livers compared to kidneys (table 1).

Groups	**n**	**Body weight**	**Liver weight**	**Kidney weight**	**Pectoral muscle weight**
C	12	201 ± 22	3.7 ± 0.6	1.3 ± 0.1	14.4 ± 1.6
		175 - 246	3.2 – 4.8	1.2 – 1.5	12.2 – 16.2
CS	6	161 ± 26	2.3 ± 0.4	2.1 ± 0.1	12.3 ± 3.2
		137 - 204	2.1 - 3.0	0.8 – 2.7	8.6 – 17.6
M	6	185 ± 14	2.9 ± 0.5	1.2 ± 0.2	14.6 ± 1.8
		162 - 211	2.1 – 3.6	1.1 – 1.4	11.6 – 16.2
MS	6	128 ± 30	2.4 ± 0.2	1.0 ± 0.1	8.0 ± 4.7
		99 - 161	2.2 – 2.6	0.9 – 1.1	3.3 – 12.3

Table 1: total body weight, liver, kidneys and pectoral muscle weight (g), expressed as mean ± standard deviation and range (min – max) in control (C group), starved control (CS group), contaminated (M group) and contaminated starved (MS group) quails.

At the end of the fasting period, severe atrophy of the pectoral muscle as well as the absence of subcutaneous and abdominal fat (cachectic status) were noted in three specimens of the contaminated group (table 2). In contrast, despite the absence of subcutaneous and abdominal fat, none of the control and starved quails developed a cachectic status.

Control group.

	Pectoral muscle volume	Subcutaneous fat	Abdominal fat	Cachexia	Subcutaneous oedema	Hepatic fatty degeneration	Other remarks
C 1	normal	yes	yes	no	no	no	-
C 2	normal	yes	yes	no	no	no	-
C 3	normal	yes	yes	no	no	no	-
C 4	normal	yes	yes	no	no	no	-
C 5	normal	yes	yes	no	no	no	-
C 6	normal	yes	yes	no	no	no	quite fat

Control and starved group.

	Pectoral muscle volume	Subcutaneous fat	Abdominal fat	Cachexia	Subcutaneous oedema	Hepatic fatty degeneration	Other remarks
CS 1	normal	no	no	no	yes	no	small testes
CS 2	normal	no	no	no	yes	no	small testes
CS 3	normal	no	no	no	yes	no	-
CS 4	normal	no	no	no	yes	no	-
CS 5	normal	no	no	no	yes	no	-
CS 6	normal	yes	yes	no	no	no	quite lean

Table 2: pathological observations in control (C group), starved control (CS group). Abnormal observations are shown in a box.

Contaminated group (Cu, Zn and CH_3Hg).							
	Pectoral muscle volume	Subcutaneous fat	Abdominal fat	Cachexia	Subcutaneous oedema	Hepatic fatty degeneration	Othe remar
M 1	normal	no	no	no	no	no	smal anim
M 2	normal	yes	yes	no	no	no	smal anima little amour of fa
M 3	normal	no	no	no	no	no	smal anim
M 4	normal	yes	yes	no	no	no	little amour of fa
M 5	normal	no	no	no	no	no	little amour of fa
M 6	normal	yes	yes	no	no	no	little amour of fa

Contaminated and starved group (Cu, Zn and CH_3Hg).							
	Pectoral muscle volume	Subcutaneous fat	Abdominal fat	Cachexia	Subcutaneous oedema	Hepatic fatty degeneration	Othe remar
MS 1	Severe atrophy	no	no	yes	yes	no	-
MS 2	normal	no	no	no	yes	no	-
MS 3	Severe atrophy	no	no	yes	yes	no	-
MS 4	Severe atrophy	no	no	yes	yes	no	-
MS 5	normal	no	no	no	yes	no	-
MS 6	normal	no	no	no	no	no	sma teste

Table 2 (continued): pathological observations in control contaminated (M group) and contaminated starved (MS group) quails. Abnormal observations are shown in a box.

Hepatic and renal heavy metal levels detected in both groups prior and after the starvation period follow a general increasing trend (figure 5):

- although not significantly different, fasted control quails displayed increased hepatic and renal Cu and Zn concentrations compared to non starved control specimens;

- a significant increase of renal Cu and Hg levels is noted for fasted contaminated individuals compared to contaminated but non fasted quails (both $p < 0.01$). Although not significantly different, hepatic Cu and Hg levels also follow an increasing trend. Hepatic Zn level remained similar in both groups while renal Zn concentrations also tend to increase for the contaminated and fasted group;

- all three metals levels remained unchanged between groups in the pectoral muscle;

- hepatic and renal Cu and Zn total loads remained unchanged in both groups. On the contrary, both Cu and Zn pectoral muscle total loads significantly decrease in contaminated fasted quails compared to contaminated non fasted individuals. For Hg, while hepatic and renal total load slightly increases, the pectoral muscle total load tends to decrease in contaminated fasted quails.

As mentioned above, three out of the six quails which were both contaminated and fasted developed a cachectic status (table 2). Comparing heavy metal levels in those animals to those observed for the non cachectic ones was of major interest. However, due to small sample size, statistical analysis could not be performed. Interestingly, a general pattern pointing towards increasing heavy metal levels in the cachectic individuals is noted with higher Cu and Zn levels in liver, kidneys and pectoral muscle while Hg concentrations tend to increase in liver and kidneys but not in the pectoral muscle (figure 6). Similarly, hepatic and renal Cu and Hg total loads tend to increase. Hepatic total Zn load remains unchanged in both cachectic and non cachectic specimens while its renal total load slightly increases. On the opposite, a general decreasing trend of the total loads was observed for all three metals in the pectoral muscle.

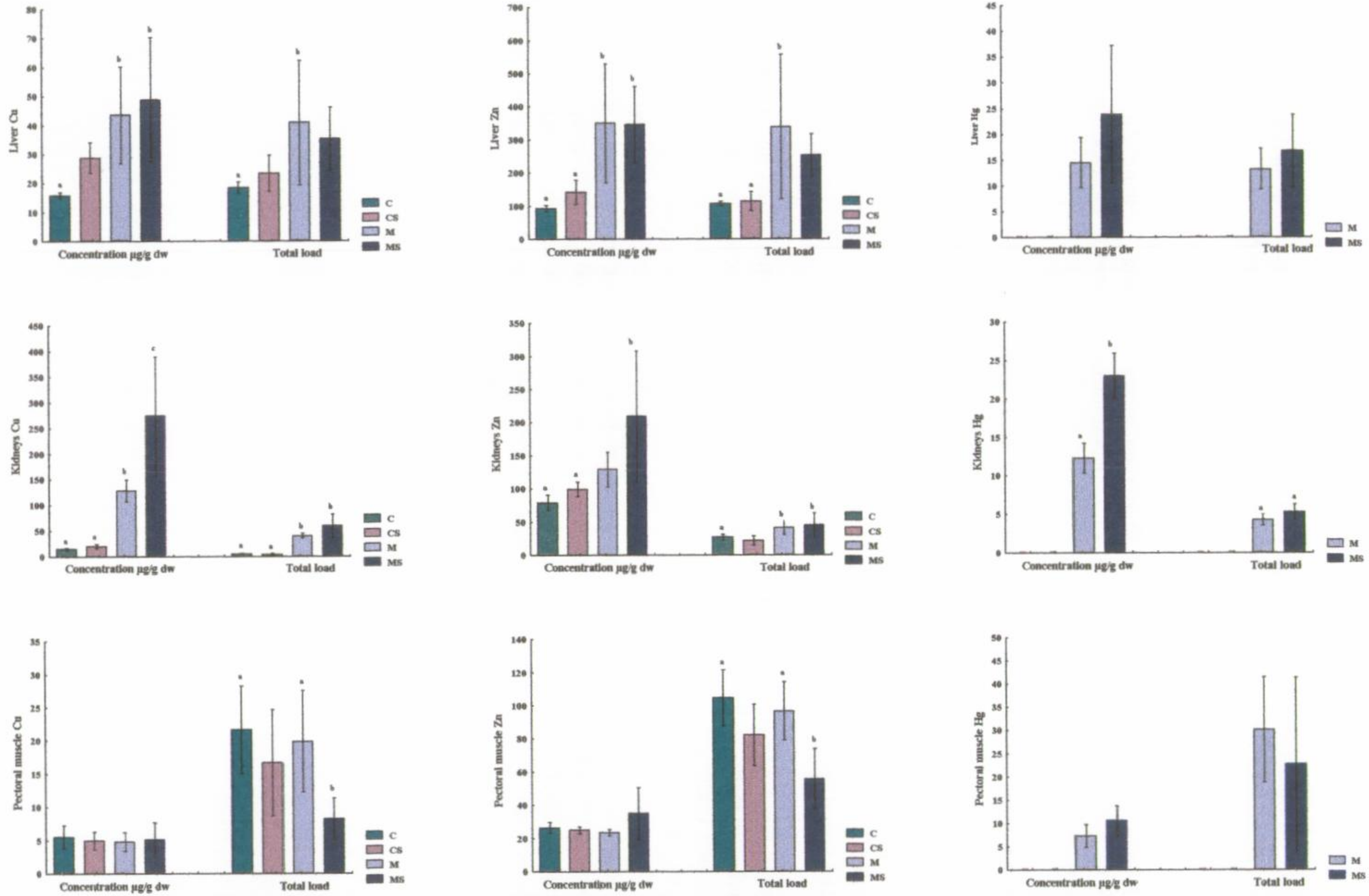


Figure 5: hepatic, renal and pectoral muscle concentrations (µg/g dw) and total loads (µg) of Cu, Zn and total Hg in control (C group), starved control (CS group), contaminated (M group) and contaminated starved (MS group) quails. Data which do not share the same superscript are significantly different from one another ($p <$ 0.05)

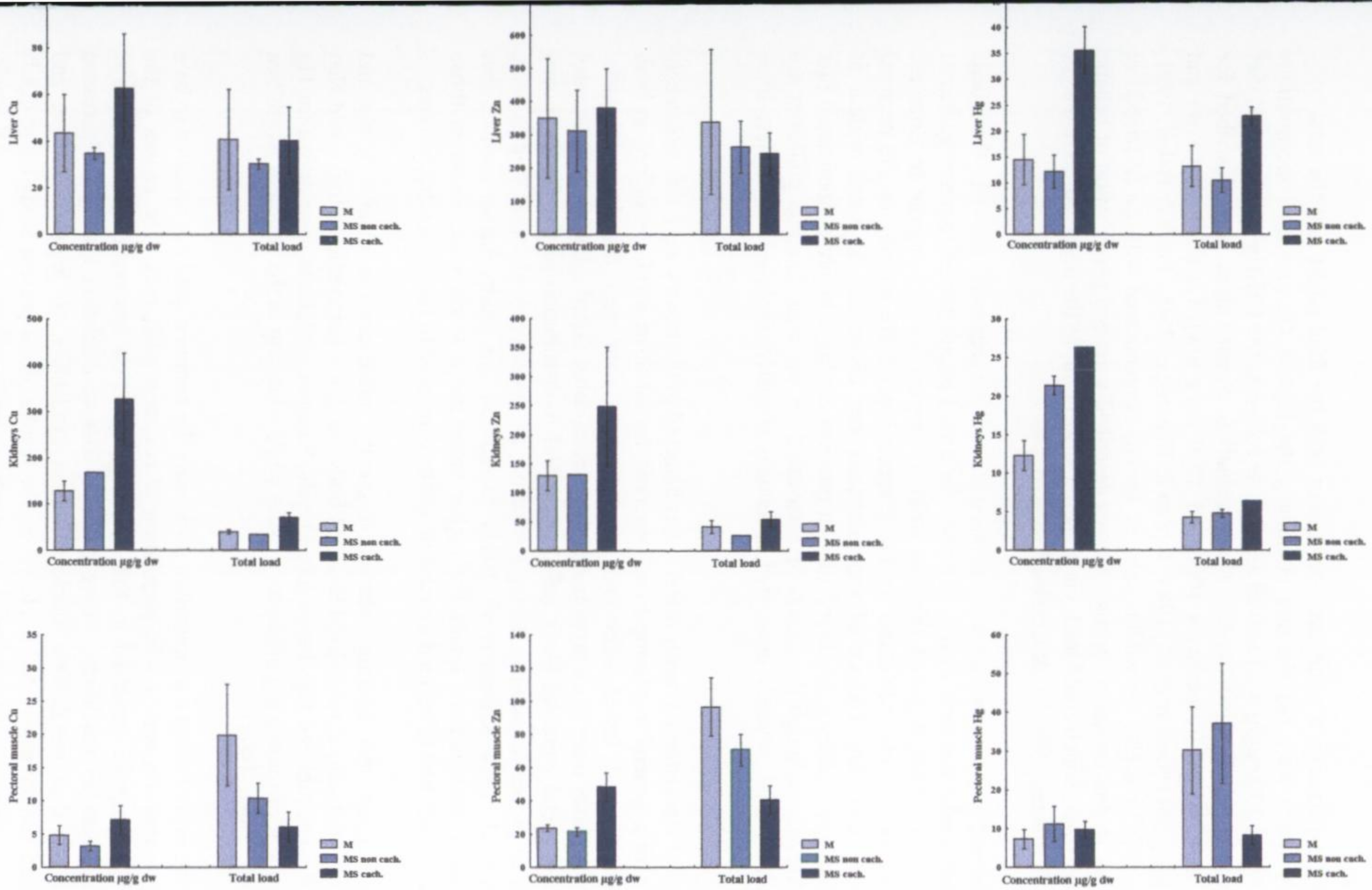


Figure 6: hepatic, renal and pectoral muscle concentrations (µg/g dw) and total loads (µg) of Cu, Zn and total Hg in contaminated (M group), contaminated starved which had not developed a cachectic status (MS non cach. group) and contaminated starved which had developed a cachectic status (MS cach. group) quails.

Discussion

During the first experiment, a significant decrease of both the food intake and the total body weight of quails which were fed the diet containing the highest Cu and Zn concentrations (respectively 600 and 3000 µg/g ww) as well as of those receiving the CH_3Hg contaminated diet (2 µg/g ww) was noted after two weeks of contamination. Similar signs were described for chicken fed an enriched Zn (respectively 1000 and 2000 µg/g ww), Cu (200 µg/g ww) and CH_3Hg diet (8 µg/g ww) (Dewar *et al.*, 1983; Jordan & Bhatnagar, 1990; Funk & Baker, 1991; Sandoval *et al.*, 1998) but also for wildlife species, heavily contaminated with Zn (Zdziarski *et al.*, 1994). Further studies on heavy metals toxicosis described a general perturbation of hepatic and pancreatic enzymes, which could lead, via unpaired absorption, to the observed body weight loss (Jordan & Bhatnagar, 1990; Lü & Combs, 1988a,b).

During the second experiment, once contaminated and subsequently starved, the quails developed evident similarities with what is described for a wild population of common guillemot (Debacker *et al.*, 1997, 2000, *in press*), the most striking common feature being the development of a cachectic status in some individuals. In those specimens, severe atrophy of the pectoral muscle as well as complete absence of sub-cutaneous and abdominal fat were evident at necropsy. Interestingly, cachexia developed only in quails receiving a highly contaminated diet and thereafter starved. Although the causes of emaciation for the wild common guillemot are likely to be multifactorial, severely emaciated specimens similarly displayed the highest trace element levels.

Marked effects of starvation on heavy metal levels detected in the tissues were also noticeable for the quails, with a general increasing trend observed for all three metals examined in both control and contaminated fasted individuals compared to their non fasted counterparts. However, these increases were more pronounced for contaminated fasted quails than for control fasted ones. These metal increases likely reflect a general re-distribution of the tissues' heavy metal levels due to protein catabolism during starvation, in agreement with other studies using quails (Richards *et al.*, 1987; Krämer *et al.*, 1993). In addition, our results further outlined that among those fasted contaminated quails the higher mean metal levels were found in those particular animals which had developed a cachectic status compared to the non cachectic ones.

These results suggest that although starvation greatly influences the metals' levels and distribution within the body, a generalized lessened body condition (cachectic status) could also be favoured by the encountered high heavy metal levels. Similar conclusions were drawn for Hg and organochlorine levels noted in guillemots' tissues while wintering in the southern North Sea (Joiris *et al.*, 1997; Tapia, 1998).

To conclude, the experimental contamination tests using the common quail has revealed to be a useful tool for comparison with a wild population of common guillemots. The causes of the severe emaciation symptom observed in the seabirds population are certainly multifactorial ranging from bad weather conditions, oiling problem, loss of insulation leading to increased energy demand and thus metabolism, decreased food availability, to impaired feeding and probable increased contaminants uptake. However, the results of the present study using quails tend to show that, although cachexia linked to starvation clearly influenced heavy metal levels, those high level encountered could well, in turn, be active participants favouring a generalized lessened body condition.

Acknowledgements

This study was financed by the 'Impulse Programme in Marine Sciences supported by the Belgian state - The Prime Minister's Services - Scientific, Technical and Cultural Affairs. (MN/DD/50 and MN/DD/51). The authors wish to thank F. Marcq and L.Duwez for providing the facilities and offering their veterinary assistance during the experiments, but also R. Biondo for his valuable technical assistance.

References

BOUQUEGNEAU, J-M. (1973). Etude de l'intoxication par le mercure d'un poisson téléostéen *Anguilla anguilla*. I. Accumulation du mercure dans les organes. Bulletin de la Société Royale des Sciences de Liège 9-10: 440-446 (1973).

DEBACKER, V., HOLSBEEK, L., TAPIA, G., GOBERT, S., JOIRIS, C.R., JAUNIAUX, T., COIGNOUL, F., BOUQUEGNEAU, J.-M. (1997). Ecotoxicological and pathological studies of common guillemots *Uria aalge* beached on the Belgian coast during six successive wintering periods (1989-90 to 1994-95). Diseases of Aquatic Organisms 29: 159-168.

DEBACKER, V., JAUNIAUX, T., COIGNOUL, F., BOUQUEGNEAU, J.-M. (2000). Heavy metals contamination and body condition of wintering guillemots (*Uria aalge*) at the Belgian coast from 1993 to 1998. Environmental Research A84, *in press*.

DEWAR, W.A., WIGHT, P.A.L., PEARSON, R.A., GENTLE, M.J. (1983). Toxic effects of high concentration of zinc oxyde in the diet of the chicks and laying hens. Poultry Science 24: 397-404.

FUNK, M.A. & BAKER, D.H. (1991). Toxicity and tissue accumulation of copper in chicks fed casein and soy-based diets. Journal of Animal Science 69: 4505-4511.

HATCH R.W. & OTT, W.L. (1968). Determination of sub-microgram quantities of mercury by atomic absorption spectrophotometry. Analytical Chemistry 40: 2085-2087.

JAUNIAUX, T., BROSENS, L., FARNIR, F., MANTECA, C., LOSSON, B., TAVERNIER, J., VINDEVOGEL H., COIGNOUL, F. (1996). Mortalité des oiseaux marins lors de l'hiver 1992-1993 le long du littoral belge. Annales de Médecine Vétérinaire 140: 149-159.

JOIRIS, C. R., TAPIA, G., HOLSBEEK, L. (1997). Increase of organochlorines and mercury levels in common guillemots *Uria aalge* during winter in the Southern North Sea. Marine Pollution Bulletin 34: 1049-1057.

JORDAN, S.A. & BHATNAGAR, M.K. (1990). Hepatic enzyme activity after combined administration of methylmercury, lead and cadmium in the Pekin duck. Bulletin of Environmental Contamination and Toxicology 44: 623-628.

KRÄMER, K., MARKWITAN, A., MENNE, A., PALLAUF, J. (1993). Zinc metabolism in fasted rats. Journal of Trace Elements and Electrolytes in Health and Disease 7: 141-146.

LŨ, J. & COMBS, G.F. (1988a). Effects of excess dietary zinc on pancreatic exocrine function in the chick. Journal of Nutrition 118: 681-689.

LŨ, J. & COMBS, G.F. (1988b). Excess dietary zinc decreases tissue *α*-tocopherol in chicks. Journal of Nutrition 118: 1349-1359.

QUEVAUVILLER, P. (1997). The QUASIMEME Project from a European Union Viewpoint. Marine Pollution Bulletin 35 (1-6): 140-145.

RICHARDS, M.P., ROSEBROUGH, R.W., STEELE, N.C. (1987). Effects of starvation and refeeding on tissue zinc, copper and iron in turkey poults. Journal of Nutrition 117: 481-489.

SANDOVAL, M., HENRY, P.R., LUO, X.G., LITTELL, R.C., MILES, R.D., Ammerman, C.B. (1998). Performance and tissue zinc and metallothionein accumulation in chicks fed a high dietary level of zinc. Poultry Science 77: 1354-1363.

STEWART, F.M., THOMPSON, D.R., FURNESS, R.W., HARRISSON, N. (1994). Seasonal variation in heavy metal levels in tissues of common guillemots, *Uria aalge* from Northwest Scotland. Archives of Environmental Contamination Toxicology 27: 168-175.

TAPIA, G.R. (1998). Mercury and organochlorines in the common guillemot *Uria aalge* wintering in the southern North Sea. Ph.D Thesis, Faculty of Medecine and Pharmacy, Free University of Brussels, Belgium.

WENZEL, C. & GABRIELSEN, G.W. (1995). Trace element accumulation in three seabird species from Hornøya, Norway. Archives of Environmental Contamination Toxicology 29:198-206.

ZDZIARSKI, J.M., MATTIX, M., BUSH, R.M., MONTALI, R.J. (1994). Zinc toxicosis in diving ducks. Journal of Zoo and Wildlife Medecine 25 (3): 438-445.

Chapter 6

Influence of age, sex and body condition on Zn, Cu, Cd and metallothioneins in common guillemots (*Uria aalge*) stranded at the Belgian coast.

Based on the manuscript by: V. Debacker[1], L-S. Schiettecatte[1], T. Jauniaux[2] and J-M. Bouquegneau[1].

[1] Oceanology, University of Liège, B6c Sart Tilman, B-4000, Belgium.

[2] Pathology Department of the Veterinary College, University of Liège, B43 Sart Tilman, B-4000, Belgium.

Revised version submitted to Marine Environmental Research

Contents

Influence of age, sex and body condition on Zn, Cu, Cd and metallothioneins in common guillemots (*Uria aalge*) stranded at the Belgian coast.

Abstract

The common guillemots, *Uria aalge*, found stranded at the Belgian coast, display high levels of Cu in both liver and kidneys. The condition index of the animals, defined as the ratio of liver to kidneys mass (Wenzel & Adelung, 1996), influences both the metal concentration and its binding to metallothioneins (MT): the lower the condition index, the more emaciated the animals, and the higher the total Cu concentration and the concentration of Cu bound to MT. In less robust individuals, our results suggest that Cu could displaces Zn from MT rendering the Zn ions available to induce a new MT synthesis. Sex related effects also emerged as significantly higher hepatic MT as well as Cu- and Zn-MT concentrations were found in emaciated male guillemots compared to females. In both organs, Cd concentrations remained low and typically demonstrated an age-dependent renal accumulation, with no noticeable effect of the condition index. As a whole, these results suggest that, for guillemots found stranded at the Belgian coast, Cu binding to hepatic and renal MT could function as a protective mechanism, rendering the metal ions unavailable to exert any cytotoxic activity.

Introduction

Studies referring to avian metallothionein (MT) analysis and using metal contaminated domestic birds are much more numerous than those referring to wild species collected in the field. In these later cases, MT analysis in seabirds has often been carried out during global toxicological investigations analysing the heavy metal levels in the environment. Hutton (1981) described a metal-binding protein from the kidney cytosol of the great skua *Catharacta skua* which bound the greater part of the cadmium (56 % of the total kidney content) and was coincident with a steep rise in absorbance at 250 nm (Cd-SH- group). Elliott and co-workers (1992) and Elliott & Scheuhammer (1997) studied metals and MT in free-living seabird species respectively from the Atlantic and Pacific coast of Canada. In both studies, the authors demonstrated a strong positive correlation between kidney cadmium and MT levels, supporting the hypothesis of increasing MT production parallel to renal cadmium accumulation. Similar conclusions were drawn by Stewart and co-workers (1996) studying heavy metal and MT concentrations in adult lesser black-backed gulls, *Larus fuscus*, collected in England and Scotland. On the contrary, for fledgling Cory's shearwaters, *Calonectris diomedea*, collected at the Azores, the same authors suggested that MT rather functioned as a Zn and Cu store, as the birds were growing plumage for which high Zn levels are required. A MT-like protein was described by Cosson (1989) in the hepatic and renal cytosol of Greater Flamingos (*Phaenicopterus ruber*) and Little Egrets (*Egretta garzetta*) wintering in the salt marshes of Camargue, France in 1985. Positive correlations between MT concentrations and hepatic copper-zinc or renal copper-zinc and cadmium levels were also shown for these species. In the common guillemots *Uria aalge*, collected stranded at the Belgian coast, Bouquegneau and co-workers (1996) demonstrated that most of, but by far not all, the additional copper ions were bound to cytosolic MT, probably as a result of the great affinity of the metal for MT compared to cadmium and zinc. Recently, Trust and co-workers (2000) conducting a study on contaminant exposure and biomarker responses in spectacled eiders (*Somateria fischeri*) observed that despite the elevated cadmium, copper, and selenium concentrations found in the organs, MT levels were quite lower than expected and were not correlated to the metals. The authors suggested that the MT induction mechanism of these eiders might not be as efficient as in other marine birds. However, MT contents in the tissues may vary depending on the species, its physiological state and ecotoxicological history or following a combination of several of these factors (Cosson & Amiard, 1998).

A large proportion of the common guillemots stranded at the Belgian coast were found in a severe body condition (Jauniaux *et al.*, 1996; Jauniaux *et al.*, 1998). Severe emaciation (cachectic status), thought of as a major cause of death, is a complex debilitating process, which, when added to other stressors (oiling, cold temperatures, feeding impairment, parasites, diseases and infections, contaminants exposure..) may be the starting point of important body disturbances. These disturbances range from metabolic (Shapiro & Weathers, 1981; Heath & Dufty, 1998) and immune disorders (Briggs *et al.*, 1996) to increased susceptibility to infectious diseases and parasites (Daoust *et al.*, 1998; Klasing, 1998) and to mineral and organic pollutants (Lemly, 1993; Esselink *et al.*, 1995; Borch-Iohnsen *et al.*, 1996; Wenzel & Adelung, 1996; Jørgensen *et al.*, 1999; Wheeler & Gates, 1999) as described for several land-based and marine wildlife species (fishes, birds and mammals). Increasing Cu and Zn concentrations were clearly linked to an increasing cachectic status for guillemots collected at the Belgian coast (Debacker *et al.*, 1997; Debacker *et al.*, 2000, *in press*). In addition, these individuals displayed higher heavy metals levels compared to individuals collected at the same period in more preserved areas like the Scottish colonies (Stewart *et al.*, 1994). However, these levels are similar to those described by Wenzel & Adelung (1996) for guillemots collected in the more polluted German Bight. The aim of the present paper is the study of the speciation of these metals, and more specifically their possible binding to metalloproteins such as metallothioneins, proteins considered as playing a key role in metal detoxification and homeostasis. Apart from heavy metal induction, metallothionein synthesis is known to be induced by a variety of factors like certain hormones but also by various conditions of physical and physiological stress, including starvation (Hidalgo *et al.*, 1990; Bremner, 1991; Kägi, 1993; McNamara & Buckley, 1994; Jacob *et al.*, 1999). In this context, relating our results to the bird's general body condition presented an opportunity to investigate different aspects of metal dynamics under stressful conditions.

Material and methods

Collection

From winters 1989-90 to 1997-98, 267 common guillemots, *Uria aalge*, were collected stranded at the Belgian coast and made available for toxicological investigations. For this study, metallothionein analysis was carried out on the livers (n =32) and kidneys (n = 14) of these individuals which were collected as follow: winter 1993-94: n= 15, winter 1994-95: n = 3, winter 1995-96: n = 1, winter 1996-97: n = 1, winter 1997-98: n = 12. All were necropsied according to a standardized protocol (Jauniaux *et al.*, 1996). The individuals were aged as immatures (<1 year old) or sub-adult to adult (potential but not necessarily breeders) based on the presence of the cloacal bursa (Camphuysen & van Franeker, 1992). Sex, total body weight, liver and kidney weight, as well as the presence of oil, **a**cute **h**aemorrhagic **g**astro-**e**nteropathy (GEAH) and other lesions were recorded during necropsy. Emaciation was evaluated and given a range from 0 to 3, depending on its severity (presence or absence of subcutaneous fat, light to severe atrophy of the pectoral muscles): specifically: 0: presence of subcutaneous fat, normal pectoral muscle; 1: absence of fat and slight pectoral muscle atrophy; 2: moderate pectoral muscle atrophy; 3: severe pectoral muscle atrophy. Body condition was also evaluated using a condition index as proposed by Wenzel & Adelung (1996). This condition index is the liver to kidneys masses ratio and is significantly negatively correlated to the cachectic status of the bird: the smaller the condition index, the more cachectic the bird (Debacker *et al.*, 2000, *in press*). Organs were collected (typically liver, kidney and pectoral muscle) and kept frozen (-18° C) prior to toxicological analyses.

Metallothioneins analysis

For each individual, a liver and/or kidney sample of 3 to 4 grams fresh weight was homogenized mixed with a buffer solution 0.01 Mol amonium formiate (pH = 7.4) containing 10 mMol sodium azide and dithithreitol 0.01 %, using an Ultra-Turrax, and centrifuged at 26000 g (60 min, 4°C). The supernatant was then filtered on an AcA 54 gel column at 4°C. Fractions were collected and absorbance profile read at 215, 250 and 280 nm using a spectrophotometer (Beckman DU® 530). After adding nitric acid (65%) to each fraction as well as part of the pellet, homogenate and supernatant, all were slowly heated to 100°C until complete digestion. Samples were diluted using deionized water and filtered prior to heavy metals analysis (Zn, Cu and Cd) by atomic absorption spectrophotometry (ARL 3510). Cd concentrations in the chromatographic fractions of liver samples were below the detection limits (< 0.18 µg/g) and have thus not been used in the further discussion. Detection limits for Cu and Zn were, respectively 0.18 and 0.17 µg/g. Concentrations are expressed as µg/g wet weight (ww.). The quality of the analyses was controlled through a participation in an intercalibration program (Quevauviller, 1997). Recovery rates ranged from 97 to 100 % for Cu and Zn respectively, and 102 % for Cd. Calculations were made assuming a MT molecular weight of 6800 Da typically binding 7 bivalent metal ions (Hamer, 1986; Kägi, 1993; Kojima *et al.*, 1999).

Lipids analysis

Lipids were extracted in liver and muscle tissues using a solvent mixture (Folch *et al.*, 1957) of methanol/chloroform (2:1, v:v) followed by purification of the extracts with a KCl solution and several dehydration steps using methanol and absolute ethanol, as described by Barnes & Blackstock (1973).

Data analysis

Statistical analysis of the data was performed using SAS statistical package (SAS Institute, Cary, NC, USA, version 6.12, 1996). Kolmogorov-Smirnov tests were used to check if the variables fitted a normal distribution. When not normally distributed the variables were log-transformed to normalize their distribution and analyses were made using parametric tests. A multiple regression analysis considering for each individual, its age, sex, condition index and metals distribution was used. Analysis of variance (Anova) was performed to compare the different groups of guillemots. The results were considered as significant at the 5 % level ($p < 0.05$) and highly significant at the 1 % level ($p < 0.01$).

Results

Condition index and heavy metals

The condition index of the guillemots (n = 162) collected along the Belgian coast during four successive wintering seasons (1993-94 to 1997-98) varied from 1.23 to 3.25, which is in the range described by Wenzel & Adelung (1996) for the same species, except one individual which displayed an unusually high condition index of 6.3 due to a large liver and very small kidneys (38.2 and 6.1 g for the liver and kidney mass, respectively). This animal also exhibited a general hepatic congestion, which could explain the organ's large mass compared to birds of similar body weight. For this reason, this guillemot was excluded from further analyses of data.

The condition index was shown to negatively correlate with Cu, Zn and Cd levels in the kidneys and with Cu and Cd levels in the livers (figure 1). A strong significative positive relation was observed between the condition index and the total lipid contents of the pectoral muscle, with declining muscle lipid contents as the body condition worsens. Such a relation is not valid for the total liver lipids for which no significant decrease is noted with decreasing condition index (figure 1: d).

Age, sex and condition index effects

Among these 162 individuals, 32 were analysed for their hepatic metallothionein content. In addition, renal MT analysis were performed on 14 of these individuals. For these guillemots, results of both MT and metal concentrations (Cu, Zn and Cd) as well as the metals distribution on MT in the organs were then statistically treated considering the individuals' age, sex and condition index entered in a multiple linear regression analysis (table1). None of these parameters were significantly determinant when considering the total Cu or Zn concentrations (µg/g ww) in neither liver or kidneys. However, hepatic concentrations of both metals bound to MT (Cu- and Zn-MT µg/g ww) and their respective percentage bound to the thionein ([Cu-MT/Tot.Cu] % and [Zn-MT/Tot.Zn] %) significantly varied following the condition index. In addition, hepatic Zn-MT and its percentage bound to MT were also significantly sex related. Variation of the hepatic MT concentration (µmole/g ww) was significantly related to both the sex and the condition index, while the hepatic MT total load (µmole) was only sex related.

For the kidneys, the condition index was significantly determinant for both Cu-MT concentration and its percentage bound to the thionein. A different situation appeared for renal Zn as only its percentage bound to MT varied significantly when considering both the sex and condition index of the bird. For renal Cd, interestingly, significant variation of total Cd and Cd-MT concentrations was both age related with no influence of either the sex nor the condition index.

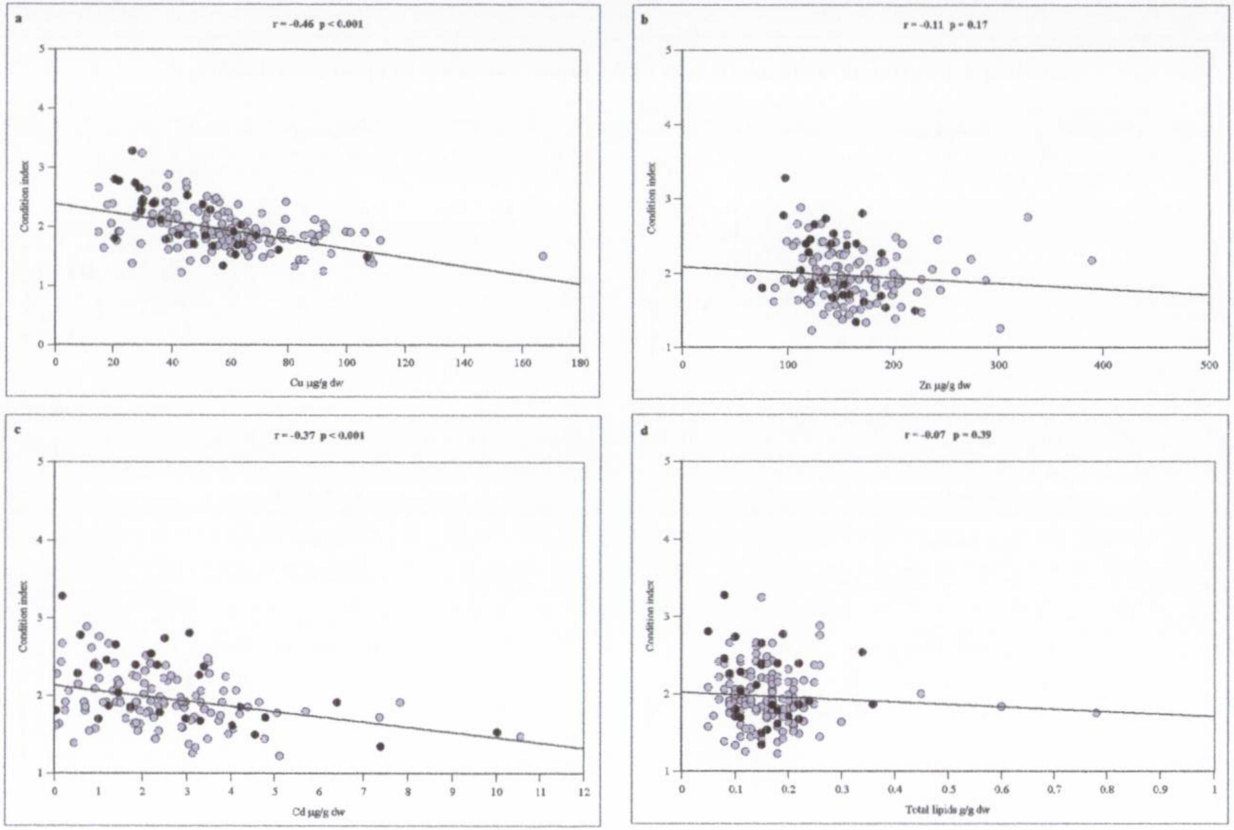


Figure 1 (a, b, c, and d): condition index versus Cu, Zn, Cd and total lipids in the livers.

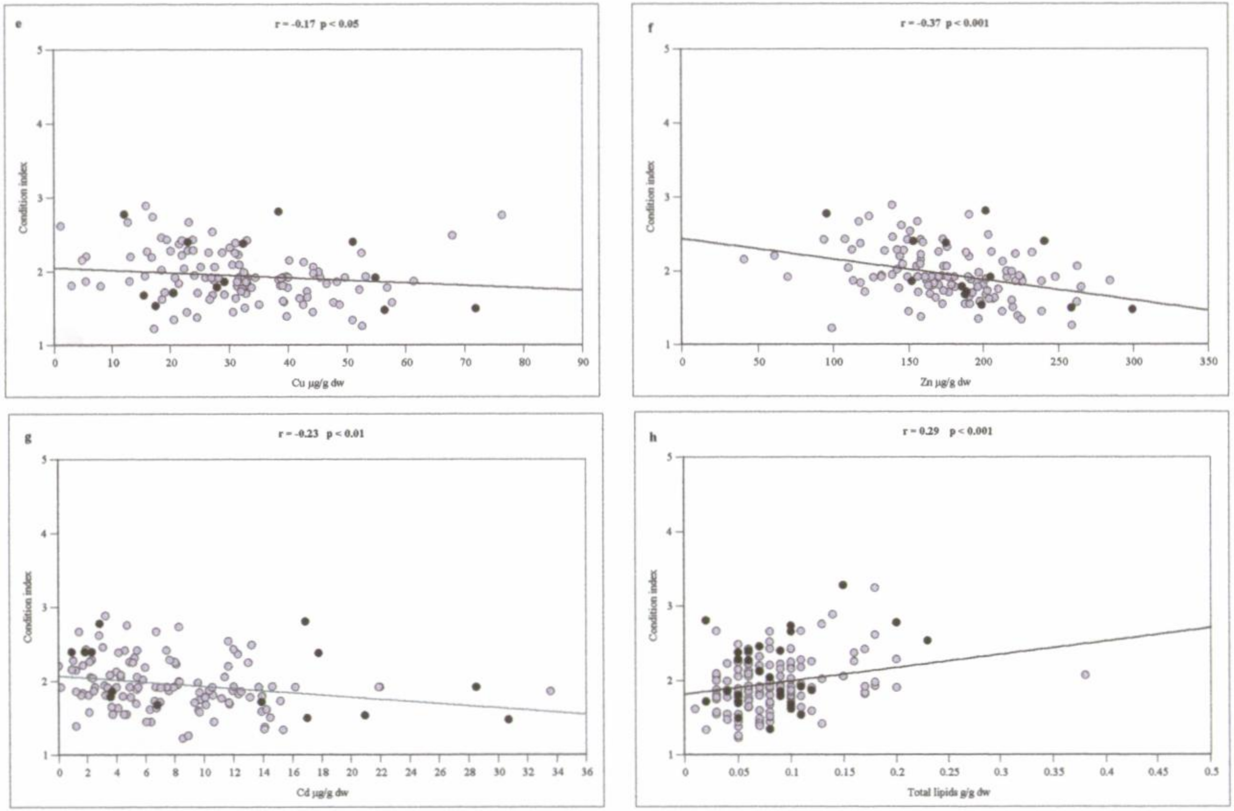


Figure 1 (e, f, g and h): condition index versus Cu, Zn and Cd in the kidneys and total lipids in the pectoral muscles.

	Age	Sex	Condition index
	LIVER		
Total Cu µg/g ww.	$p = 0.29$	$p = 0.29$	$p = 0.08$
Cu-MT µg/g ww.	$p = 0.54$	$p = 0.08$	$p < 0.01$
$\frac{\text{Cu-MT}}{\text{Cu Tot.}}$ %	$p = 0.90$	$p = 0.13$	$p < 0.01$
Total Zn µg/g ww.	$p = 0.70$	$p = 0.41$	$p = 0.76$
Zn-MT µg/g ww.	$p = 0.97$	$p < 0.01$	$p < 0.05$
$\frac{\text{Zn-MT}}{\text{Zn Tot.}}$ %	$p = 0.76$	$p < 0.01$	$p < 0.01$
MT µmole/g ww.	$p = 0.38$	$p < 0.05$	$p < 0.01$
MT µmole	$p = 0.66$	$p < 0.05$	$p = 0.50$
	KIDNEYS		
Total Cu µg/g ww.	$p = 0.06$	$p = 0.95$	$p = 0.07$
Cu-MT µg/g ww.	$p = 0.08$	$p = 0.92$	$p < 0.05$
$\frac{\text{Cu-MT}}{\text{Cu Tot.}}$ %	$p = 0.35$	$p = 0.91$	$p < 0.05$
Total Zn µg/g ww.	$p = 0.09$	$p = 0.72$	$p = 0.99$
Zn-MT µg/g ww.	$p = 0.53$	$p = 0.31$	$p = 0.10$
$\frac{\text{Zn-MT}}{\text{Zn Tot.}}$ %	$p = 0.18$	$p < 0.05$	$p < 0.01$
Total Cd µg/g ww.	$p < 0.05$	$p = 0.94$	$p = 0.53$
Cd-MT µg/g ww.	$p < 0.05$	$p = 0.72$	$p = 0.35$
$\frac{\text{Cd-MT}}{\text{Cd Tot.}}$ %	$p = 0.63$	$p = 0.49$	$p = 0.27$
MT µmole/g ww.	$p = 0.26$	$p = 0.45$	$p = 0.07$
MT µmole	$p = 0.35$	$p = 0.11$	$p = 0.12$

Table 1: multiple linear regression analysis considering the age, sex, and condition index. Trace elements were envisaged as total concentration in the organs (µg/g ww), as bound to MT (Cu-, Zn- and Cd-MT, µg/g ww) and as a percentage bound to MT ([Cu-MT/Tot.Cu] %; [Zn-MT/Tot.Zn] % and [Cd-MT/Tot.Cd] %). MT total load in the organs (µmole) is also given.

Metallothionein and condition index

The heavy metal speciation, through binding to MT, was thus studied considering two groups of guillemots: those – emaciated to varying degrees - with a condition index $\leqslant 2$, and those –

more robust - with a condition index > 2, as suggested by Wenzel & Adelung (1996) who considered that guillemots with a condition index ⩽ 2 were in bad physiological conditions when compared to healthy guillemots collected alive from Arctic colonies. In our study, the liver of those emaciated guillemots significantly lost weight compared to more robust specimens (table 2).

LIVER	**Condition index**		**Statistical comparison**	
n = 31	> 2 n = 14	⩽ 2 n = 17	$F_{1,29}$	p
Liver weight (g)	27.04 ± 6.82	16.85 ± 2.33	33.38	$p < 0.001$ ↘↘↘
Total Cu µg/g ww	12.0 ± 4.0	17.5 ± 12.0	2.67	$p = 0.11$
Cu-MT µg/g ww	3.8 ± 2.5	7.6 ± 4.0	9.61	$p < 0.01$ ↗↗
$\frac{\text{Cu-MT}}{\text{Cu Tot.}}$ %	29.9 ± 16.7	45.0 ± 11.1	9.03	$p < 0.01$ ↗↗
Total Cu µg	314.4 ± 92.9	288.5 ± 166.6	0.27	$p = 0.61$
Cu-MT µg	101.4 ± 71.6	126.2 ± 59.5	1.12	$p = 0.30$
Total Zn µg/g ww	45.9 ± 14.0	44.0 ±11.0	0.18	$p = 0.67$
Zn-MT µg/g ww	10.1 ± 5.1	12.8 ± 6.5	1.62	$p = 0.21$
$\frac{\text{Zn-MT}}{\text{Zn Tot.}}$ %	21.5 ± 6.8	27.8 ± 10.8	3.58	$p = 0.07$
Total Zn µg	1220.2 ± 447.2	751.7 ± 254.8	13.43	$p < 0.001$ ↘↘↘
Zn-MT µg	275.1 ± 175.2	217.5 ± 118.0	1.19	$p = 0.28$
MT µmole/g ww	0.028 ± 0.01	0.044 ± 0.02	6.50	$p < 0.05$ ↗
MT µmoles	0.73 ± 0.35	0.74 ± 0.34	0.001	$p = 0.97$
Percentage of Cu and Zn bound to MT $\frac{\mu\text{mole metal}}{7\text{x } \mu\text{mole MT}} \times 100$				
% Cu	25.2 ± 12.8	37.9 ± 13.8	6.94	$p < 0.05$ ↗
% Zn	74.8 ± 12.8	62.1 ± 13.8	6.94	$p < 0.05$ ↘

Table 2: one-way ANOVA testing the influence of the condition index on liver weight (g), total trace elements (Cu and Zn) and MT concentrations (µg/g ww) and total loads (µg). Heavy metals binding to MT (Cu- and Zn-MT µg/g ww) and total loads (Cu- and Zn-MT µg) as well as

their percentage bound to the thionein ([Cu-MT/Tot.Cu] %; [Zn-MT/Tot.Zn] %) are given. Results are expressed as mean ± standard deviation. Those for which the comparison was significant are shown with arrows (coefficient F and p level given: ↘: $p < 0.05$; ↘↘ : $p < 0.01$; ↘↘↘ : $p < 0.001$).

In the liver, while not significant, the total Cu concentration tends to increase with decreasing condition index. In addition, hepatic Cu-MT concentration significantly increased for emaciated individuals while the percentage of Cu bound to the MT increased by a 1.5 factor (respectively 29.9 and 45.0 %). For Zn, a rather different situation appeared as similar total Zn concentrations were observed in both groups while the total Zn load (µg), significantly decreased with decreasing body condition. Simultaneously, the Zn-MT concentration tends to increase (1.27 times more Zn-MT) in emaciated birds, as well as its percentage bound to MT.

Another important component of this analysis was to evaluate the relative distribution of both elements (Cu and Zn) when stored on MT. Assuming that, in the liver, only Zn and Cu are bound to MT in significant amount, it appeared that the percentage of Cu relative to the total amount of metals bound to MT, increased with degradating body condition, while the percentage of Zn simultaneously decreased.

In the kidneys (table 3), a similar trace elements distribution pattern appeared: both total Cu concentration and load tend to increase with decreasing condition index. Both Cu-MT concentration and its percentage bound to MT significantly increased for guillemots with lowest condition index. Renal Zn-MT concentration, while not significantly different, also tend to increase in the same group. A rather different situation appeared for Cd, for which the age (figure 2), and not the condition index, significantly determined its distribution on MT.

KIDNEYS	**Condition index**		**Statistical comparison**	
n = 14	> 2 n = 7	⩽ 2 n = 7	$F_{1,12}$	p
Kidneys weight (g)	11.30 ± 1.85	9.40 ± 1.66	4.19	$p = 0.06$
Total Cu µg/g ww	8.0 ± 2.6	16.0 ± 12.5	2.71	$p = 0.13$
Cu-MT µg/g ww	1.8 ± 1.2	5.8 ± 4.5	5.06	$p < 0.05$ ↗
$\frac{\text{Cu-MT}}{\text{Cu Tot.}}$ %	20.9 ± 11.3	35.5 ± 5.9	9.17	$p < 0.01$ ↗↗
Total Cu µg	89.6 ± 30.5	150.4 ± 121.5	1.64	$p = 0.22$
Cu-MT µg	20.7 ± 14.6	54.5 ± 43.8	3.74	$p = 0.08$

Total Zn µg/g ww	51.0 ± 15.3	52.6 ± 15.8	0.04	p = 0.84
Zn-MT µg/g ww	13.1 ± 5.6	17.9 ± 7.2	1.94	p = 0.19
$\frac{\text{Zn-MT}}{\text{Zn Tot.}}$ %	25.3 ± 7.2	33.7 ± 8.5	3.94	p = 0.07
Total Zn µg	568.4 ± 197.4	500.0 ± 180.6	0.47	p = 0.51
Zn-MT µg	146.2 ± 69.2	172.7 ± 83.3	0.42	p = 0.53
Total Cd µg/g ww	2.38 ± 1.52	3.41 ± 2.53	0.85	p = 0.37
Cd-MT µg/g ww	1.53 ± 1.11	2.41 ± 1.78	1.21	p = 0.29
$\frac{\text{Cd-MT}}{\text{Cd Tot.}}$ %	59.4 ± 21.4	68.8 ± 14.7	0.92	p = 0.36
Total Cd µg	26.7 ± 18.3	32.9 ± 24.2	0.30	p = 0.60
Cd-MT µg	16.9 ± 12.9	23.7 ± 17.8	0.66	p = 0.43
MT µmole/g ww	0.026 ± 0.01	0.055 ± 0.03	3.18	p = 0.09
MT µmole	0.39 ± 0.19	0.53 ± 0.28	1.26	p = 0.28
	Percentage of Cu, Zn and Cd bound to MT		$\frac{\text{µmole metal}}{\text{7x µmole MT}} \times 100$	
% Cu-MT	11.9 ± 5.1	22.1 ± 7.6	8.62	p < 0.01 ↗
% Zn-MT	83.1 ± 6.5	72.9 ± 8.0	6.91	p < 0.05 ↘
% Cd-MT	5.0 ± 2.7	5.0 ± 2.6	0.001	0.99

Table 3: one-way ANOVA testing the influence of the condition index on kidneys weight (g), total trace elements (Cu and Zn) and MT concentrations (µg/g ww) and total loads (µg). Heavy metals binding to MT (Cu- and Zn-MT µg/g ww) and total loads (Cu- and Zn-MT µg) as well as their percentage bound to the thionein ([Cu-MT/Tot.Cu] %; [Zn-MT/Tot.Zn] % and [Cd-MT/Tot.Cd] %) are given. Results are expressed as mean ± standard deviation. Those for which the comparison was significant are shown with arrows (coefficient F and p level given: ↘: $p < 0.05$; ↘↘ : $p < 0.01$; ↘↘↘ : $p < 0.001$).

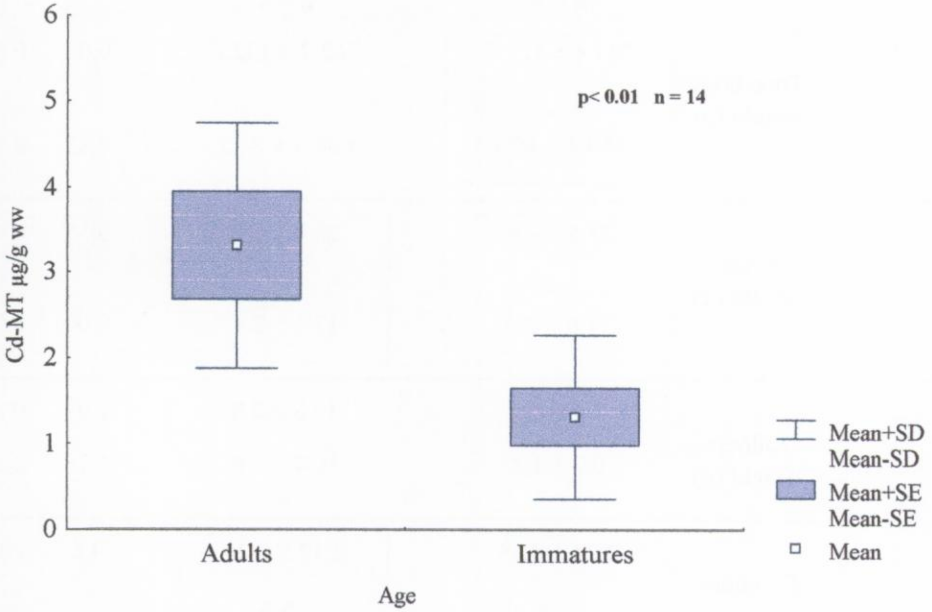


Figure 2: difference of renal Cd accumulation between adult and immature (< 1 year old) guillemots.

Sex effects

As mentioned above sex related effects emerged from the multiple regression analysis, most often combined with the condition index (table 1). For this reason, the effects of these two determinant factors were statistically tested using one-way Anova (table 4). Twenty-nine guillemots for which the sex was known were considered in the analysis. Emaciated male guillemots displayed significantly higher hepatic Cu and Zn-MT concentrations compared to emaciated females. Cu- and Zn-MT hepatic total load as well as the percentage of Zn-MT in the organ also followed the same distribution. In addition, both hepatic MT concentration and total load were shown to significantly increase for emaciated male birds. Interestingly, no significant differences were noted between robust males and females. Other significant variations are also reported, testing either robust against emaciated females or robust against emaciated males. These variations, however, reflected a decrease of the guillemot's condition index and were not sex related.

Condition index		Female n = 5 n = 9	Male n = 9 n = 6	F	p
> 2	Total body weight (g)	781.4 ± 125.7	762.7 ± 132.0	0.07	0.80
		↘			
⩽ 2		659.3 ± 80.1	698.3 ± 84.2	0.82	0.38
> 2	Liver weight (g)	25.8 ± 4.6	28.8 ± 8.2	0.55	0.47
		↘↘	↘↘		
⩽ 2		17.0 ± 2.6	17.1 ± 2.4	0.00	0.96
> 2	Kidneys weight (g)	9.4 ± 0.9	10.8 ± 2.8	1.04	0.33
⩽ 2		9.7 ± 1.4	10.7 ± 1.6	1.78	0.20
> 2	Condition index	2.74 ± 0.35	2.42 ± 0.20	4.6	0.054
		↘↘	↘↘↘		
⩽ 2		1.77 ± 0.19	1.60 ± 0.15	3.04	0.10
		Liver			
> 2	Total Cu µg/g ww	13.1 ± 4.7	11.2 ± 3.6	0.83	0.38
			↗		
⩽ 2		12.9 ± 4.2	24.6 ± 18.1	3.56	0.08
> 2	Cu-MT µg/g ww	3.4 ± 3.2	3.7 ± 2.2	0.05	0.83
			↗↗		
⩽ 2		5.4 ± 2.1 ↗↗	10.6 ± 4.7	8.53	0.01
> 2	Cu-MT / Cu Tot. %	22.4 ± 14.9	32.8 ± 16.5	1.42	0.25
		↗↗			
⩽ 2		41.0 ± 8.2	49.0 ± 14.4	1.91	0.19

> 2	Total Cu µg	337.5 ± 123.8		304.4 ± 72.6	0.44	0.52
≤ 2		225.9 ± 92.1		392.8 ± 232.2	3.86	0.07
> 2	Cu-MT µg	85.4 ± 76.9		106.3 ± 68.7	0.29	0.60
≤ 2		93.0 ± 41.1	↗↗	173.5 ± 57.3	10.12	0.01
> 2	Total Zn µg/g ww	48.1 ± 17.5		45.6 ± 12.3	0.11	0.75
≤ 2		41.6 ± 12.0		51.2 ± 4.2	3.48	0.09
> 2	Zn-MT µg/g ww	9.2 ± 5.8		10.6 ± 4.6	0.25	0.63
				↗↗↗		
≤ 2		9.4 ± 8.0	↗↗↗	19.3 ± 2.2	20.43	0.001
> 2	Zn-MT / Zn Tot. %	17.9 ± 6.6		23.0 ± 6.1	2.23	0.16
				↗↗↗		
≤ 2		21.7 ± 7.8	↗↗↗	38.0 ± 5.7	19.05	0.001
> 2	Total Zn µg	1216.8 ± 393.4		1303.6 ± 535.3	0.10	0.75
		↘				
≤ 2		724.0 ± 296.7		875.3 ± 139.2	1.34	0.27
> 2	Zn-MT µg	224.9 ± 130.7		311.8 ± 187.9	0.85	0.37
≤ 2		160.6 ± 94.9	↗↗	331.0 ± 60.7	15.0	0.01
> 2	MT µmole/g ww	0.028 ± 0.02		0.027 ± 0.01	0.003	0.96
				↗↗↗		
≤ 2		0.032 ± 0.01	↗↗	0.063 ± 0.02	13.32	0.01

> 2	MT µmole	0.68 ± 0.45		0.78 ± 0.30	0.26	0.62
⩽ 2		0.56 ± 0.27	↗↗	1.05 ± 0.21	13.57	0.01

Percentage of Cu and Zn bound to MT $\frac{\underline{\mu mole\ metal}}{7x\ \mu mole\ MT} \times 100$

> 2		25.1 ± 8.9	24.2 ± 14.6	0.02	0.90
	% Cu	↗			
⩽ 2		38.3 ± 10.6	31.9 ± 15.6	0.90	0.36
> 2		74.9 ± 8.9	75.8 ± 14.6	0.02	0.90
	% Zn	↘			
⩽ 2		61.7 ± 10.6	68.1 ± 15.6	0.90	0.36

Table 4: one-way ANOVA testing both the sex and condition index effects, with horizontally: comparison between robust females and males and emaciated females and males (coefficient F and p level given), and vertically: comparison between robust and emaciated females and robust and emaciated males (coefficient F and p level not given). Results are expressed as a mean ± standard deviation. Those for which the comparison was significant (↘: $p < 0.05$; ↘↘ : $p < 0.01$; ↘↘↘ : $p < 0.001$) are shown in a box.

Discussion

Guillemots at the Belgian coast have been shown to carry higher Cu and Zn concentrations in their organs compared to other specimens collected in more preserved areas of the North Sea (Debacker *et al.*, 1997). However, these higher levels are closely related to a decrease of their condition index probably following a moderate to severe starvation process (Debacker *et al.*, 2000, *in press*). During this process, fat reserves available as energy are depleted before the organism turns to its protein reserves as an energy source. Our results showed a significant decrease of the muscle lipid contents, indicating their progressive use while facing starvation. On the contrary, the liver is known to be a key site for lipid metabolisation during the early stages of starvation (Saudek & Felig, 1976; Le Maho *et al.*, 1981; Cherel *et al.*, 1988; Herzberg *et al.*, 1988), which could explain the apparent lack of significant decrease of hepatic lipid contents with decreasing condition index.

When considering MT and their possible role as a detoxifying mechanism, our results indicated that a decreasing condition index significantly affected the hepatic and renal metals distribution on the protein. More specifically, although not significant, higher total hepatic Cu concentrations are observed in emaciated birds, probably resulting from the increased general metabolism leading to protein consumption. Simultaneously, our results showed a significant increase of both the hepatic Cu-MT concentration as well as its percentage bound to the thionein in emaciated guillemots, clearly indicating that most of that additional Cu is bound to MT. These results are in agreement with a previous study about heavy metals and MT analysis carried out on guillemots collected in the early 1990s at the Belgian coast, which demonstrated that most of the additional Cu was bound to cytosolic MT (Bouquegneau *et al.*, 1996). However, for Zn, a rather different situation appeared as similar total Zn concentration are described for both groups, while the total Zn load, as a result of the organ weight loss, significantly decreased with decreasing body condition. Krämer and co-workers (1993) working on rats described a similar significant depletion of total hepatic Zn in starved animals. Under such conditions, the urinary Zn excretion is known to increase with concurrent reduction of the total body Zn store (Beisel *et al.*, 1976; Cousins, 1985). Our results further indicated that 1.27 times more Zn-MT was found in emaciated animals, which results from a significantly increased hepatic MT concentration (figure 3) rather than from an increased number of Zn ions bound to each MT molecule. However, due to the organ weight loss, similar MT total load was observed in both groups.

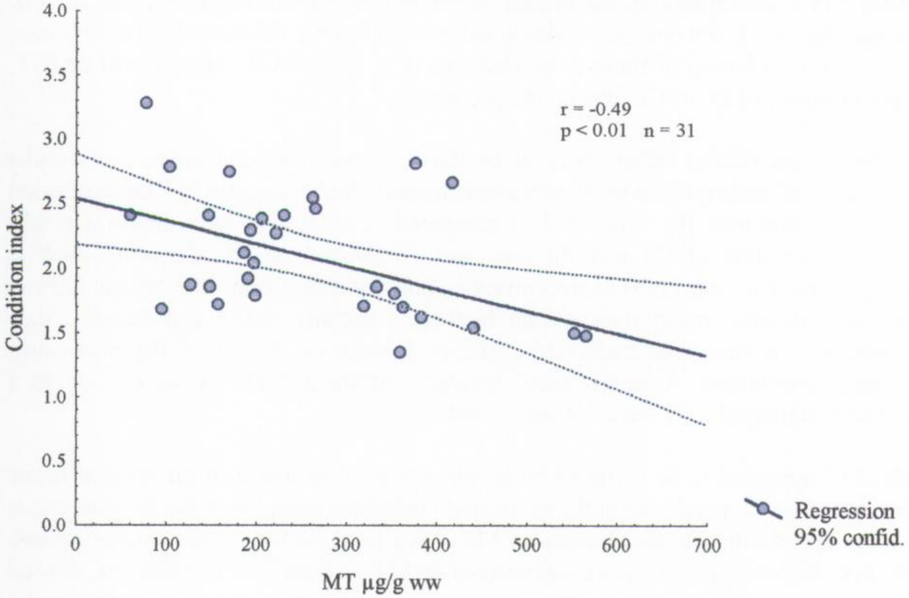


Figure 3: condition index versus MT concentration in the livers.

The relative distribution of both elements (Cu and Zn) when stored on MT was also investigated. Assuming that, in the liver, only Zn and Cu are bound to MT in significant amount, it appeared that the percentage of Cu relative to the total amount of metals bound to MT, increased with degradating body condition, while the percentage of Zn simultaneously decreased. This results either from Zn excretion during starvation, either from a substitution of Zn by the additional Cu ions on MT (rendering the released Zn ions available to induce a new MT synthesis), or both. Cu has a higher affinity for –SH groups than Zn and once bound to MT, is thus less easily released (Kägi, 1993; Viarengo & Nott, 1993). Recent studies have described MT to function as a transporter of essential trace elements whose metal binding and release capacity are regulated in a redox sensitive manner (Maret *et al.*, 1997; Maret & Vallee, 1998; Jacob *et al.*, 1998; Jiang *et al.*, 1998; Zhang *et al.*, 1999). More specifically for Cu, Fabisiak and co-workers (1999) demonstrated that once bound to MT, Cu redox activity is reduced and thus renders the metal less available to participate in a variety of redox cycling reactions that could give rise to several reactive oxygen species and thus potentiate cellular damages. In this view, MT is a an important sequestering protein offering cytoprotective effects during metal exposure and oxidative stress.

Kidney displayed a similar Cu accumulation pattern: both total Cu concentration and load tend to increase with decreasing condition index. This could be attributed to a general increased metabolism induced during starvation periods rather than the organ weight loss as, unlike the liver, the kidneys lose very little weight with degradating body condition. Both Cu-MT concentration and percentage significantly increased for guillemots with lowest condition index, suggesting that additional Cu ions are taken up on MT in these individuals. Renal Zn-MT concentration, while not significantly different, also tends to increase for the same group, a result of the increasing MT concentration in the organs. A rather different situation appeared for Cd, for which the age (figure 3), and not the condition index, significantly determined its distribution on MT. The relative distribution of these three elements (Cu, Zn and Cd) when stored on MT, suggested a displacement of Zn by Cu ions on the protein.

When considering sex related effects parallel to those of the condition index, our results indicated that emaciated male guillemots displayed significantly higher hepatic MT concentration (by a factor 2) and total load (by a factor 1.9) compared to emaciated females. As a result, significantly higher amounts of Cu and Zn ions were mobilized on the thionein which is demonstrated by higher Cu- and Zn-MT concentrations noted in this group. The hepatic Cu and Zn-MT total loads, despite similar liver weight between emaciated males and females, thus significantly increased in emaciated male birds. The percentage of Zn-MT in the organ also followed the same distribution. A similar trend emerged for the kidneys, however, due to a smaller sample size, statistical tests were not performed.

To conclude, MT appeared to be involved in the physiological response to the general heavy metals homeostasis disruption induced in the emaciated guillemots stranded at the Belgian coast. Metals, and more specifically Cu distribution on MT, have been shown to vary following two distinct levels: first, higher levels of Cu are sequestered on MT, in both liver and kidneys, as total Cu concentrations increased; second: in both organs, MT bound a higher percentage of Cu and a lower percentage of Zn per µmole of MT, suggesting a displacement of Zn by the additional Cu ions. In this context, MT synthesis could be viewed as a general response to stressing conditions as it appears to offer a cytoprotective mechanism against free Cu ions toxicity. It is however worth noting that, in our sample, this response was clearly sex related as emaciated male guillemots displayed higher MT as well as Cu- and Zn-MT concentrations.

Acknowledgements

This study was financed by the 'Impulse Programme in Marine Sciences supported by the Belgian state - The Prime Minister's Services - Scientific, Technical and Cultural Affairs.' (MS/12/032, MS/12/033, MN/DD/50 and MN/DD/51). The authors wish to thank C. Daemers-Lambert for her critical reading of earlier drafts which undoubtedly improved the manuscript. We thank P. Meire, H. Offringa, J. Seys, J. van Waeyenberg, W. Roggeman, J. Tavernier for collecting and providing the specimens and R. Biondo for his valuable technical assistance.

References

BARNES, H. & BLACKSTOCK, J. (1973). Estimation of lipids in marine animals and tissues: detailed investigation of the sulphosphovanillin method for 'total' lipids. Journal of Experimental Marine Biology and Ecology 12: 103-118.

BEISEL, W.R., PEKAREK, R.S., WANNEMACHER, R.W.Jr. (1976). Homeostatic mechanisms affecting plasma zinc levels in acute stress. In: Prasad, A.S. & Oberleas, D. (Eds). Trace elements in human health and disease. Volume II. Essential and toxic elements. Academic Press, London, 87-106.

BORCH-IOHNSEN, B., NILSSEN, K.J., NORHEIM, G. (1996). Influence of season and diet on liver and kidney content of essential elements and heavy metals in Svalbard reindeer. Biological Trace Element Research 51: 235-247.

BOUQUEGNEAU J-M., DEBACKER V., GOBERT S. & HAVELANGE S. (1996). Role of metallothioneins in metal regulation by the guillemot *Uria aalge*. Comparative Biochemistry and Physiology 113 C: 135-139.

BREMNER, I. (1991). Nutritional and physiological significance of metallothionein. Methods in Enzymology 205: 25-35.

BRIGGS, K.T., YOSHIDA, S.H., GERSHWIN, M.E. (1996). The influence of petrochemicals and stress on the immune system of seabirds. Regulatory Toxicology and Pharmacology 23: 145-155.

CAMPHUYSEN C.J. & VAN FRANEKER J.A. (1992). The value of beached bird surveys in monitoring marine oil pollution. Technisch Rapport Vogelbescherming 10. Vogelbescherming Nederland, Zeist, 191 pp.

CHEREL, Y., ROBIN, J-P., LE MAHO, Y. (1988). Physiology and biochemistry of long term fasting in birds. Canadian Journal of Zoology 66: 159-166.

COSSON, R.P. (1989). Relationships between heavy metal and metallothionein-like protein levels in the liver and kidney of two birds: the Greater Flamingo and the Little Egret. Comparative Biochemistry and Physiology 94 C *(1)*: 243-248.

COSSON, R.P. & AMIARD, J.C. (1998). Utilisation des métallothionéines comme biomarqueurs d'exposition aux métaux. In: Lagadic, L., Caquet, T., Amiard, J.C., Ramade F. (Eds). Utilisation de biomarqueurs pour la surveillance de la qualité de l'environement. Lavoisier Tec&Doc, Paris, 77-109.

COUSINS, R.J. (1985). Absorption, transport and hepatic metabolism of copper and zinc: special reference to metallothioneins and ceruloplasmin. Physiological Reviews 65 *(2)*: 238-308.

DAOUST, P-Y., CONBOY, G., MCBURNEY, S., BURGESS, N. (1998). Interactive mortality factors in common loons from maritime Canada. Journal of Wildlife Diseases 34 (3): 524-531.

DEBACKER, V., HOLSBEEK, L., TAPIA, G., GOBERT, S., JOIRIS, C.R., JAUNIAUX, T., COIGNOUL, F., & BOUQUEGNEAU, J.-M. (1997). Ecotoxicological and pathological studies of common guillemots *Uria aalge* beached on the Belgian coast during six successive wintering periods (1989-90 to 1994-95). Diseases of Aquatic Organisms 29: 159-168.

DEBACKER, V., JAUNIAUX, T., COIGNOUL, F., & BOUQUEGNEAU, J.-M. (2000). Heavy metals contamination and body condition of wintering guillemots (*Uria aalge*) at the Belgian coast from 1993 to 1998. Environmental Research, *in press*.

ELLIOTT, J.E., & SCHEUHAMMER, A.M. (1997). Heavy metal and metallothionein concentrations in seabirds from the Pacific coast of Canada. Marine Pollution Bulletin 34 (10*)*:794-801.

ELLIOTT, J.E., SCHEUHAMMER, A.M., LEIGHTON, F.A., PEARCE, P.A. (1992). Heavy metal and metallothionein concentrations in Atlantic Canadian seabirds. Archives of Environmental Contamination and Toxicology 22: 63-73.

ESSELINK, H., VAN DER GELD, F.M., JAGER, L.P., POSTHUMA-TRUMPIE, G.A., ZOUN, P.E.F., BAARS, A.J. (1995). Biomonitoring heavy metals using the Barn Owl (*Tyto alba guttata*): sources of variation especially relating to body condition. Archives of Environmental Contamination and Toxicology 28:, 471-486.

FABISIAK, J.P., TYURIN, V.A., TYURINA, Y.Y., BORINSENKO, G.G., KOROTAEVA, A., PITT, B.R., LAZO, J.S., KAGAN, V.E. (1999). Redox regulation of copper-metallothionein. Archives of Biochemistry and Biophysics 363 (1*)*: 171-181.

FOLCH, J., LEES, M., SLOANE STANLEY, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226: 497-509.

HAMER, D.H. (1986). Metallothionein. Annual Review of Biochemistry 55: 913-951.

HEATH, J.A. & DUFTY, A.M. Jr. (1998). Body condition and the adrenal response in captive American kestrel juveniles. Physiological Zoology 71 *(*1): 67-73.

HERZBERG, G.R., BROSNAN, J.T., HALL, B., ROGERSON, M. (1988). Gluconeogenesis in liver and kidney of common murre (*Uria aalge*). American Journal of Physiology 254: R903-R907.

HIDALGO, J., BORRÁS, M., GARVEY, J.S., ARMARIO, A. (1990). Liver, brain and heart metallothionein induction by stress. Journal of Neurochemistry 55: 651-654.

HUTTON, M. (1981). Accumulation of heavy metals and selenium in three seabird species from the United Kingdom. Environmental Pollution (A) 26: 129-145.

JACOB, C., MARET, W., VALLEE, B.L. (1998). Control of zinc transfer between thionein, metallothionein, and zinc proteins. Proceedings of the National Academy of Sciences of the United States of America 95: 3489-3494.

JACOB, S.T., GHOSHAL, K., SHERIDAN, J.F. (1999). Induction of metallothionein by stress and its molecular mechanisms. Gene Expression 7: 301-310.

JAUNIAUX, T., BROSENS, L., FARNIR, F., MANTECA, C., LOSSON, B., TAVERNIER J., VINDEVOGEL, H., COIGNOUL, F. (1996). Mortalité des oiseaux marins lors de l'hiver 1992-1993 le long du littoral belge. *Annales de Médecine Vétérinaire 140*, 149-159.

JAUNIAUX, T., BROSENS, L., MEIRE, P., OFFRINGA, H., COIGNOUL, F. (1998). Pathological investigations on guillemots (*Uria aalge*) stranded at the Belgian coast during the winter 1993-94. Veterinary Record 143: 387-390.

JIANG, L-J., MARET, W., VALLEE, B.L. (1998). The glutathione redox couple modulates zinc transfer from metallothionein to zinc-depleted sorbitol dehydrogenase. Proceedings of the National Academy of Sciences of the United States of America 95: 3483-3488.

JØRGENSEN, E.H., BØJRN, E.B., JOBLING, M. (1999). Influence of nutritional status on biomarker responses to PCB in the Arctic charr (*Salvelinus alpinus*). Aquatic Toxicology 44: 233-244.

KÄGI, J.H.R. (1993). Evolution, structure, and chemical activity of class I metallothioneins: an overview. In: Suzuki, K.T., Imura, N., Kimura, M., (Eds). Metallothionein III: biological role and medical implications. Birkhäuser Verlag, Basel, 29-55.

KLASING, K.C. (1998). Nutritional modulation of resistance to infectious diseases. Poultry Science 77: 1119-1125.

KOJIMA, Y., P-A. BINZ, KÄGI, J.H.R. (1999). Nomenclature of metallothionein: proposal for a revision. In: Klaassen, C.D. (Ed). Metallothionein IV. Birkhäuser Verlag, Basel, 3-6.

KRÄMER, K., MARKWITAN, A., MENNE, A., PALLAUF, J. (1993). Zinc metabolism in fasted rats. Journal of Trace Elements Electrolytes Health Diseases 7: 141-146.

LE MAHO, Y., VU VAN KHA, H., KOUBY, H., DEWASMES, G., GIRARD, J., FERRÉ, P., CAGNARD, M. (1981). Body composition, energy expenditure, and plasma metabolites in long-term fasting geese. American Journal of Physiology 241: E342-E354.

LEMLY, A. D. (1993). Metabolic stress during winter increases the toxicity of selenium to fish. Aquatic Toxicology 27: 133-158.

MARET, W., LARSEN, K.S., VALLEE, B.L. (1997). Coordination dynamics of biological zinc "clusters" in metallothioneins and in the DNA-binding domain of the transcription factor Gal4. Proceedings of the National Academy of Sciences of the United States of America 94: 2233-2237.

MARET, W., & VALLEE, B.L. (1998). Thiolate ligands in metallothionein confer redox activity on zinc clusters. Proceedings of the National Academy of Sciences of the United States of America 95: 3478-3482.

MCNAMARA, P.T., & BUCKLEY, L.J. (1994). Identification and characterization of metallothionein cDNA from mRNA transcripts induced by starvation in Atlantic cod (*Gadus morhua*). Molecular Marine Biology and Biotechnology 3 (5): 252-260.

QUEVAUVILLER, P. (1997). The QUASIMEME Project from a European Union Viewpoint. Marine Pollution Bulletin 35 (1-6): 140-145.

SAUDEK, C.D., & FELIG, P. (1976). The metabolic events of starvation. American Journal of Medecine 60: 117-126.

SHAPIRO, C.J., & WEATHERS, W.W. (1981). Metabolic and behavioral responses of American kestrels to food deprivation. Comparative Biochemistry and Physiology 68A: 11-114.

STEWART, F.M., THOMPSON, D.R., FURNESS, R.W., HARRISON, N. (1994). Seasonnal variation in heavy metal levels in tissues of common Guillemots, *Uria aalge* from Northwest Scotland. Archives of Environmental Contamination and Toxicology 27: 168-175.

STEWART, F.M., FURNESS, R.W., MONTEIRO, L.R. (1996). Relationships between heavy metal and metallothionein concentrations in Lesser Black-backed Gulls, *Larus fuscus*, and Cory's shearwater, *Calonectris diomedea*. Archives of Environmental Contamination and Toxicology 30: 299-305.

TRUST, K.A., RUMMEL, K.T., SCHEUHAMMER, A.M., BRISBIN, I.L.JR., HOOPER, M.J. (2000). Contaminant exposure and biomarker responses in spectacled eiders (*Somateria fischeri*) from St. Lawrence Island, Alaska. Archives of Environmental Contamination and Toxicology 38: 107-113.

VIARENGO, A. & NOTT, J. A. (1993). Mechanisms of heavy metal cation homeostasis in marine invertebrates. Comparative Biochemistry and Physiology 104C: 355-372.

WENZEL, C. & ADELUNG, D. (1996). The suitability of oiled Guillemots (*Uria aalge*) as monitoring organisms for geographical comparisons of trace element contaminants. Archives of Environmental Contamination and Toxicology 31: 368-377.

WHEELER, W.E. & GATES, R.J. (1999). Spatial and temporal variation in lead levels related to body condition in the Mississippi Valley population of Canada geese. Journal of Wildlife Diseases 35 (2): 178-186.

ZHANG, S., LI, J., WANG, C-C., TSOU, C-L. (1999). Metal regulation of metallothionein participation in redox reactions. FEBS Letters 462: 383-386.

General discussion and conclusions.

General discussion

Evidence of heavy metals accumulation and possible detrimental effects in wild bird species has received great attention during the last decades. A vast majority of these studies referred to robust individuals but not much research work has been devoted to the study of the potential detrimental effects of heavy metals on debilitated specimens. Stranded debilitated seabirds represent a choice material offering a wide range of conditions often closely related. They are also likely to be more susceptible to the contaminants' potential detrimental effects compared to laboratory animals as they have to face a range of other (natural and anthropogenic) stressors (Nicholson *et al.*, 1983 ; Foulkes, 1990). This study presents a first attempt to clarify the potential existing links between pathological and toxicological findings and precise the role of heavy metals as additional debilitating agents in a population of wintering common guillemots found stranded at the Belgian coast.

Stranded guillemots collected on our coasts most likely represent only a small fraction of the total population at sea during the winter as it is a known fact that not all dying birds at sea are recovered on beaches (Bibby & Lloyd, 1977; Bibby, 1981; Bodkin & Jameson, 1991; Hlady & Burger, 1993; Van Pelt & Piatt, 1995; Piatt & Ford, 1996; Flint & Fowler, 1998). Comparison with the population found wintering in the southern North sea is difficult as un unknown percentage of the individuals at sea will eventually strand on our coasts while the others either sink, are eaten by scavengers or simply move away. The individuals collected at the Belgian coast are believed to originate mainly from the northeastern Scottish colonies (W. Roggeman *pers. com.* see Appendix table 3).

All the birds analyzed in this study (from winter 1989-90 to winter 1997-98) were collected dead or moribund at the Belgian coast during the wintering season (November to early April). During the first studied wintering seasons (from winter 1989-90 to 1993-94), sampled guillemots included both birds directly collected on the beaches and those which had died following rehabilitation. These individuals had been collected still alive or moribund on the beaches and were sent to rehabilitation centres in an attempt to revalidate and later release them. Unfortunately, most of them died in the process and were subsequently made available for necropsy and toxicological analysis.

A first analysis of the sample distinctively revealed that guillemots of both origins ('beached' and 'rehabilitated') could not be further compared on the basis of their toxicological results as significantly higher levels of Zn, Fe, total and inorganic Hg and PCBs had been detected in the tissues of those rehabilitated guillemots. For this reason, from winter 1993-94, guillemots which had been treated in a rehabilitation centre prior to dying were automatically removed from the sample. Efforts then totally focused on beached birds only, even if one cannot exclude that some successfully rehabilitated specimens get once again washed ashore and enter the beached bird sample. However, the number of successful release of guillemots following rehabilitation remains much lower than the number of 'really' stranded birds during the same period.

At necropsy, among other findings, a large majority of these guillemots presented clear signs of a general emaciation characterized by a mild to severe atrophy of the pectoral muscles and complete absence of subcutaneous and/or abdominal fat deposits (cachectic status) (Appendix, photo 1 a and b). Similar observations are reported in the literature as a striking feature not only of stranded seabirds (Parslow & Jefferies, 1973 ; Hope Jones *et al.*, 1984; Camphuysen, 1989; Cunningham & Simmonds, 1992; Forrester *et al.*, 1997; Piatt & Van Pelt, 1997; Daoust *et al.*, 1998), but also of land based species (Esselink *et al.*, 1995 ; Thouzeau *et al.*, 1999a,b). It is generally assumed that glycogen stores are the first to be depleted in the early stages of starvation (the first 24 hours), then fat deposits are mobilized before the bird progressively relies on protein catabolism as an energy source. This last phase is often characterized by a higher rate of body mass loss and is usually indicative of a critical limit (Cherel *et al.*, 1988 ; Totzke *et al.*, 1999). Both the absence of subcutaneous and/or abdominal fat and the mild to severe atrophy of the pectoral muscle observed in our study, largely confirm that a large majority of the guillemots had to rely on their protein reserves as an energy source prior to dying. If we look at that cachectic status as either 'present' or 'absent', it appears that cachectic guillemots have lost up to 32.5 % of their total body weight compared to robust individuals weighing around 1 kg (table 1). However, if we take into account cachectic status according to severity (from winter 1993-94 onwards : from non cachectic : '-' to low : '+1', moderate : '+2' and severely cachectic : '+3'), our results indicate that severely cachectic individuals (cachectic '+3') have lost up to 43 % of their total body weight (chapter 3). These losses are twice as high as those reported by Herzberg (1991), who observed a body weight loss of 21.5 % in experimentally fasted guillemots after a 3-day complete starvation.

Location	Date	Weight g.	Source
Eastern Irish Sea, England. Found stranded.	Winter 1969	580 ± 14 (range 542-673) adults n = 8 (7 females and 1 sex unknown).	Parslow & Jefferies, 1973.
Moray Firth, East England Found stranded.	Winter 1983	676 (range 450-1030) adults. 629 (range 490-1000) immatures.	Hope Jones *et al.*, 1984.
The Netherlands Found stranded.	Winter 1982 -1985	718 ± 69.6 males, adults. 705.8 ± 46.7 females, adults. 724 ± 93.3 males, immatures. 673.8 ± 93.3 females, adults.	Camphuysen, 1989.
Newfoundland, Canada	Experiment 1985-1986	Before fasting: 720 ± 82 (n =4) After a 3-day fast: 565 ± 62	Herzberg, 1991.
Belgian coast Found stranded.	Winter 1989-1998	Cachectic '+' : 784.7 ± 143.4 (n = 52) Cachectic '-' : 675.2 ± 106.2 (n = 211) Adults : 711.6 ± 133.6 (n = 86) Immatures : 686.4 ± 108.3 (n = 148)	This study
Belgian coast Found stranded.	Winter 1993-1998	Cachectic '-' : 758 ± 114 (n = 23) Cachectic '+1' : 710 ± 85 (n = 31) Cachectic '+2' : 640 ± 64 (n = 79) Cachectic '+3' : 574 ± 47 (n = 29)	This study

Table 1: literature data giving body weight (g) (expressed as mean ± standard deviation or range) of guillemots found stranded at several locations.

In addition, a large majority of the guillemots examined in this study (86.4 %) did not present any food remains in their stomach, whether they showed an empty stomach (33.3 %) or clear signs of an haemorrhagic content (53.1 %). Although it is not surprising, it is worth noting that most of these individuals were also cachectic (table 2). Food remains were found in 20 specimens (12.3 %) and always consisted of partially digested items. In no case were fresh fish pieces recovered. Intestinal dysfunction (altered epithelial transport) has been shown to increase in malnourished rats (Darmon *et al.*, 1993) while evidence of the atrophy of the digestive tract (significant decrease of structural lipid contents) in parallel to starvation have been demonstrated in wildlife bird species (*Podiceps nigricollis*, Jehl, 1997; *Tyto alba*, Thouzeau *et al.*, 1999b). Such changes might well have negative effects on the digestive's tract integrity (favouring invading pathogens) and digestive function (Klasing, 1997).

	Intestinal contents				
Cachexia	Empty	Blood	Normal	Unknown	Total
'-'	10	9	3	1	**23**
'+1'	15	13	3	0	**31**
'+2'	24	47	8	0	**79**
'+3	5	17	6	1	**29**
Total	**54**	**86**	**20**	**2**	**162**

Table 2: frequency distribution of the observed intestinal content in relation to cachexia severity.

In this context, we first examined the heavy metal levels in the tissues of cachectic versus non cachectic guillemots, the former clearly displaying higher renal and hepatic Cu, Zn, total and inorganic Hg and PCBs levels (chapter 2). These differences became even more striking when taking into account the different categories attributed to the cachectic status (from non cachectic : '-' to low : '+1', moderate : '+2' and severely cachectic : '+3'): the more cachectic the bird the higher the Cu and Zn levels found in the tissues. In addition, when statistically testing the potential influence of different variables (explanatory variables : sex, age, presence or absence of oiling, presence or absence of **a**cute **h**emorrhagic **g**astro**e**nteropathy - GEAH - and cachexia severity) on heavy metal levels (response variables), using a multiple regression analysis, the cachectic status has been shown to significantly affect hepatic, renal and pectoral muscle Cu and Zn concentrations (chapter 3).

In response to combined environmental stressors (*e.g.* cold weather, starvation, oiling..), the guillemot's body and organs weight were shown to significantly decrease in cachectic individuals. In particular, the liver lose much more weight than the kidneys while among muscles, the pectoral muscles are the first to be depleted. As a result of this general weight loss, trace elements (in this case Cu and Zn) concentrations significantly increase in the tissues. When considering the organ's total metal load, no such trend was observed except in the case of Cu total loads, which significantly increased in kidneys and decreased in pectoral muscles respectively. Despite the tissues'weight loss, total lipid burdens of both liver and pectoral muscles also decreased significantly ($p<0.001$ and $p< 0.0001$, respectively), underlining the use of fat reserves as energy source prior to protein catabolism. These results suggested that a general redistribution of heavy metals (Cu and Zn) in the organs is likely to occur as a result of prolonged starvation and protein catabolism.

During this whole study and on the contrary to Cu and Zn, Cd concentration in the tissues revealed to be mainly dependent of the age of the individuals, with the highest levels observed in the kidneys of adult guillemots (chapters 2, 3 and 6). Although it is generally assumed that

Cd concentration increases with age, not much is yet known on the potentially continuing accumulation of Cd with growing age. Analysis of exactly known age individuals should clarify this issue (Stewart & Furness, 1998). In our study such a distinction could not be made as the birds were aged following the presence of the cloacal bursa (see Material and methods of the chapters 2, 3 and 6) with no idea of the precise age. However, Cd levels detected in the stranded guillemots are well below the proposed treshold hepatic and renal Cd levels above which Cd poisoning might be expected (respectively of 40 and 100 ppm ww or ± 160 and 400 ppm dw) (Furness, 1996).

As the determination of the cachectic status is based on visual observations at necropsy, we also used a quantitative value as an additional tool to evaluate the guillemot's general health status. This value is the condition index or liver to kidneys weight ratio, as proposed by Wenzel & Adelung (1996). Haematological values have also been proposed as a useful tool to evaluate avian health (Jeffrey *et al.*, 1985 ; Bearhop *et al.*, 1999a,b ; Newman *et al.*, 1999 ; Seiser *et al.*, 2000). However, in this study the condition index was favoured for practical reasons. As expected, the condition index and cachectic status were negatively correlated, with lowest condition index observed for severely cachectic individuals (chapter 3).

Compared to guillemots collected in more preserved areas of the North Sea, specimens collected at the Belgian coast were shown to display higher concentrations of Cu, Zn (chapters 2 and 3) and total Hg (chapter 2). A specific study of Hg and organochlorines (total PCBs and p,p'-DDE) concentrations in the same individuals demonstrated that significantly higher levels of these contaminants were detected in the guillemots while wintering in the southern North Sea (Joiris *et al.*, 1997 ; Tapia, 1998). The authors discussed both a seasonal cyclic contamination-decontamination pattern and higher anthropogenic contaminant inputs (for Hg and organochlorines) in the southern North Sea. Although no seasonal pattern was observed for either Cu, Zn, Fe and Cd in our sample, evidence of a more contaminated southern North Sea appeared while comparing heavy metal levels between guillemots collected in Brittany (France), following Erika's oil spill to those collected at the Belgian coast (chapter 4). As body condition was shown to drastically influence heavy metal levels in the tissues (chapters 2 and 3), we compared guillemots of similar condition index and invariably found higher levels of Cu and Zn in all three tissues of the Belgian guillemots. This holds true even when comparing the more robust guillemots (condition index > 2.5) of both origins, suggesting that contaminant levels tend to be higher in the southern North Sea.

In addition to this useful geographical comparison, this Brittany sample further confirmed the process of a general heavy metal redistribution within the organ as a result of starvation and protein catabolism : once again, higher Cu and Zn levels were associated with a low condition index while more robust individuals displayed lower levels.

Given these higher Cu and Zn levels observed and although these trace elements are essential to life, the question arises to know whether or not these higher levels could have any detrimental effects and act as an additional stressor on the guillemot's general health status (chapters 5 and 6).

To answer this question, a set of experiments using the common quail (*Coturnix coturnix*) was developed to test the combined effect of contamination (using Cu, Zn and CH_3Hg) and starvation (chapter 5). Several studies demonstrated that the general pattern of the metabolic adaptative response of quails to long term fasting is similar to that observed in larger birds

(Didier *et al.*, 1981; Sartori *et al.*, 1995). Appropriate heavy metal levels were given to the quails to obtain concentrations similar to those found in the guillemot's tissues. The individuals were then completely starved during four days. A cachectic status was shown to develop in half of the quails which were both contaminated and fasted while the fasted non contaminated counterparts did not display any cachectic characteristics. In addition, higher heavy metal levels were detected in the tissues of the cachectic quails (fasted and contaminated) compared to non cachectic specimens of the same group. These results tend to suggest that, although cachexia linked to starvation clearly influenced heavy metal levels in the tissues, those encountered high levels could well, in turn, be active participants favouring a generalized lessened body condition.

In this general described context, examining the metals' speciation - and more specifically their possible binding to metallothioneins (MT) - became of interest. In particular, relating these results to the guillemots' general body condition offered an opportunity to investigate another aspect of metal dynamic under stressful conditions. Results indicated that the condition index of the guillemots clearly influenced both the metal concentration and its binding to MT, in particular for Cu : the lower the condition index, the higher the total Cu concentration and the concentration of Cu bound to MT in both liver and kidneys. In addition, in both organs, MT bound a higher percentage of Cu and a lower percentage of Zn per µmole of MT, suggesting a displacement of Zn by the additional Cu ions. These results suggest that MT synthesis could be viewed as a general response to stressing conditions for stranded guillemots, as it appears to offer a cytoprotective effect against free Cu ions toxicity.

Conclusions

While wintering in the southern North Sea, the common guillemots have to face a wide variety of natural and anthropogenic stressful conditions. Apart from oiling (acute and chronic) which remains an important indirect source of mortality, starvation has been shown to greatly influence the bird's general health status as well as its heavy metals levels. In the wild, stressful conditions are rarely isolated and most often combined to others. A general schematic view of combined stressors and their potential detrimental effects is proposed (table 3). Chemical stressors, interacting in a wide range of combination can induce a variety of sublethal tissues and organ responses and increase energy requirements and depletion of stored body lipids (Lemly, 1993, 1996).

The tolerance and responses of the common guillemots to these combined stressors will determine its ability to survive. In contrast to well adapted long-term faster species like geese (Le Maho *et al.*, 1981) and penguins (Groscolas, 1986 ; Cherel *et al.*, 1988 ; Robin *et al.*, 1998) which can starve for weeks, common guillemots seem to be able to resist and probably recover to short-term fast of a few days (Herzberg, 1991) but would probably be unable to recover a longer starving period (± 10 days of total fast, Golovkin, 1963 cited by Braedstreet & Brown, 1985). In the case of the guillemots collected stranded on the Belgian coast, we can not exclude periods of partial feeding prior dying although it seems logical that with increasing exhaustion basic activities such as foraging and feeding are impaired.

While starving and re-adjusting its whole metabolism and certainly increasing it to face its demanding energy requirements, the guillemot also undergoes a general redistribution of its Cu and Zn contents. This results in increasing circulating trace elements which are then re-routed towards target organs where they reach levels which, in turn, could well favour a generalized lessened body condition.

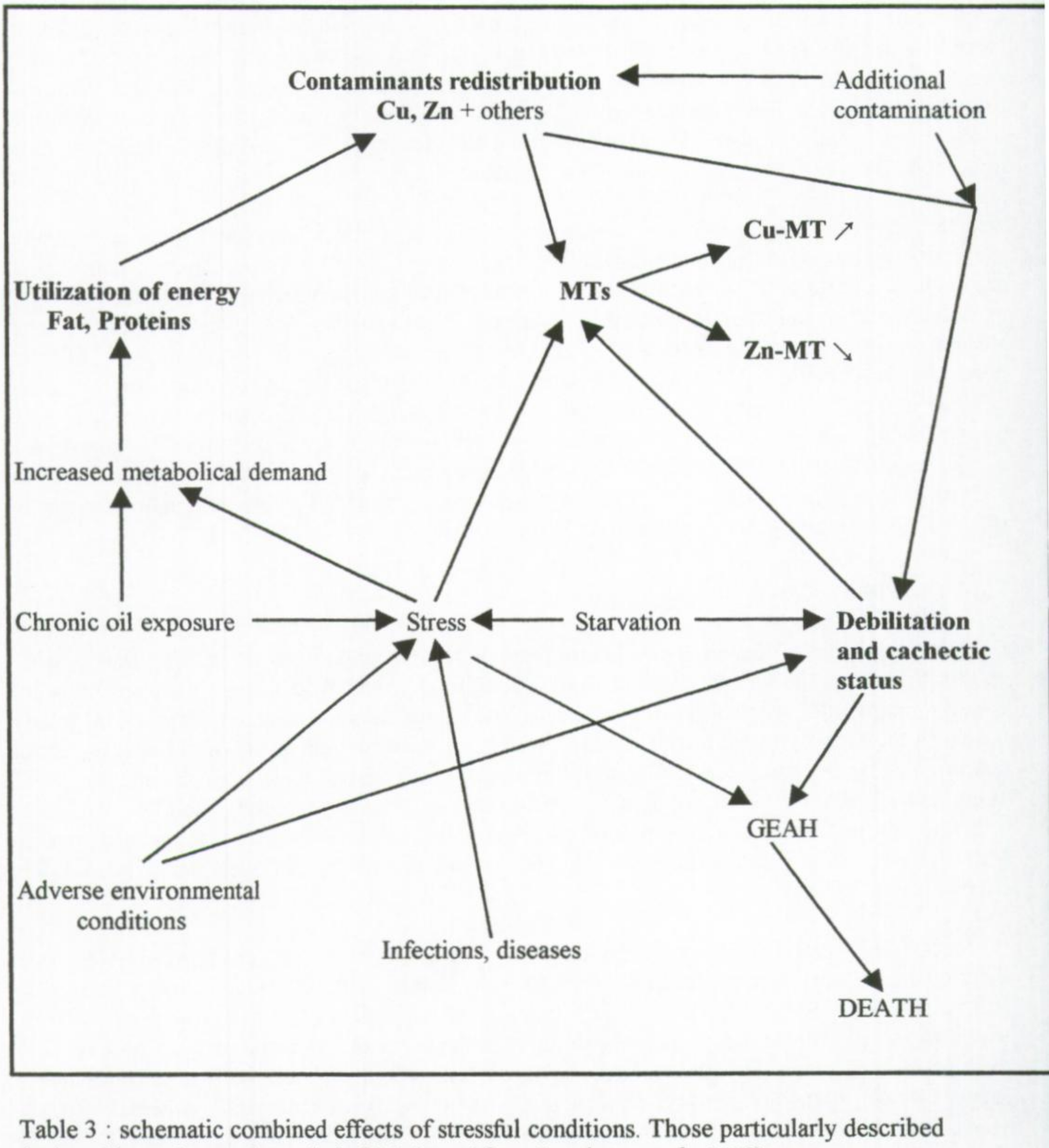


Table 3 : schematic combined effects of stressful conditions. Those particularly described during this study are shown in bold (adapted from Jauniaux *et al.*, 1998).

It is generally assumed that animals exposed to high levels of metals for a long time have evolved mechanisms to minimize the potential detrimental effects of these contaminants. However, this does not exclude the possibility that the animals are affected by the metals. Our own data on metals'speciation suggest a key role for metallothioneins. These metalloproteins appeared to be involved in the physiological response to the general heavy metal homeostasis disruption induced in the emaciated guillemots stranded at the Belgian coast. It is therefore suggested that the high proportion of Cu bound to MT for guillemots with a low condition index points towards a significant role of MT in Cu sequestration, possibly at the expense of the regulation of Zn. However, the question rises and remains debatable to know at what cost, debilitated emaciated specimens can still afford MT synthesis while consuming their protein reserves.

In view of these different points, re-assessing the role of heavy metals in a debilitated stranded population of common guillemots gave a first insight of the complex interactions existing between contaminants, their potential detrimental effects and the individuals' general fitness. Although not at risk on a toxicological basis under normal conditions (robustness) the guillemots could well be adverselly affected by their Cu and Zn levels with degradating body condition.

References

BIBBY, C.J. (1981). An experiment on the recovery of dead birds from the North Sea. Ornis Scandinavia 12 : 261-265.

BIBBY, C.J. & LLOYD, C.S. (1977). Experience to determine the fate of dead birds at sea. Biological Conservation 12 : 295-309.

BEARHOP, S., GRIFFITHS, R., ORR, K., FURNESS, R.W. (1999a). The normal haematology of great skuas (*Catharacta skua*) in the wild. Comparative Haematology International 9 : 107-109.

BEARHOP, S., GRIFFITHS, R., ORR, K., FURNESS, R.W. (1999b). Mean corpuscular volume (MCV) as a measure of condition in birds. Ecology Letters 2 : 352-356.

BODKIN, J.L. & JAMESON, R.J. (1991). Patterns of seabird and marine mammal carcass deposition along the central California coast, 1980-1986. Canadian Journal of Zoology 69: 1149-1155.

BRAEDSTREET, M.S.W. & BROWN, R.G.B. (1985). Feeding ecology of the Atlantic Alcidae. In: Nettleship, D.N. & Birkhead, T.R. (Eds). The Atlantic Alcidae: the evolution, distribution and biology of Auks inhabiting the Atlantic Ocean and adjacent waters areas. Academic Press, London, 263-318.

CAMPHUYSEN, C.J. (1989). Beached bird surveys in The Netherlands, 1915-1988. Seabird mortality in the southern North Sea since the early days of oil pollution. (Techn. Rapport Vogelbescherming 1) Werkgroep Noordzee, Amsterdam.

CHEREL, Y., ROBIN, J.-P., LE MAHO, Y. (1988). Physiology and biochemistry of long-term fasting in birds. Canadian Journal of Zoology 66 : 159-166.

CUNNINGHAM, A.A. & SIMMONDS, M. (1992). Unusual mass mortality of juvenile kittiwakes (*Rissa tridactyla*). The Veterinary Record 130:448-449.

DAOUST, P.-Y., CONBOY, G., MCBURNEY, S., BURGESS, N. (1998). Interactive mortality factors in common loons from Maritime Canada. Journal of Wildlife Diseases 34 (3): 524-531.

DARMON, N., PÉLISSIER, M-A., HEYMAN, M., ALBRECHT, R., DESJEUX, J-F. (1993). Oxidative stress may contribute to the intestinal dysfunction of weanling rats fed a low protein diet. Journal of Nutrition 123: 1068-1075.

DIDIER, R., REMESY, C., DEMIGNE, C. (1981). Effet du jeûne sur les principaux métabolites sanguins et hépatiques de la néoglucogenèse et de la cetogenèse chez la caille domestique adulte (*Coturnix coturnix japonica*). Reproduction, Nutrition and Développement 21 (3): 421-428.

ESSELINK, H., VAN DER GELD, F.M., JAGER, L.P., POSTHUMA-TRUMPIE, G.A., ZOUN, P.E.F., BAARS, A.J. (1995). Biomonitoring heavy metals using the Barn Owl (*Tyto alba guttata*): sources of variation especially relating to body condition. Archives of Environmental Contamination and Toxicology 28: 471-486.

FLINT, P.L. & FOWLER, A.C. (1998). A drift experiment to assess the influence of wind on recovery of oiled seabirds on St Paul Island, Alaska. Marine Pollution Bulletin 36(2): 165-166.

FORRESTER, D.J., DAVIDSON, W.R., LANGE, R.E.Jr., STROUD, R.K., ALEXANDER, L.L., FRANSON, J.C., HASELTINE, S.D., LITTELL, R.C., NESBITT, S.A. (1997). Winter mortality of common loons in Florida coastal waters. Journal of Wildlife Diseases 33: 833-847.

FOULKES, E.C. (1990). The concept of critical levels of toxic heavy metals in target tissues. Toxicolgy 20 (5) : 327-339.

FURNESS, R.W. (1996). Cadmium in birds. In: Beyer, W.N., Heinz, G.H., Redmon-Norwood, A.W. (Eds). Environmental contaminants in wildlife: interpreting tissue concentrations. CRC Press, 389-404.

GOLOVKIN, A.N. (1963). Murres (*Uria* species) and Black-legged kittiwakes (*Rissa tridactyla*) fish consumption during the nesting period in the Barents Sea. Zoologicheskii Zhurnal 42 (3): 408-416. (Translated from Russian, 1983: Doenv Tr-2287, Environment Canada, Ottawa).

GROSCOLAS, R. (1986). Changes in body mass, body temperature and plasma fuel levels during the natural breeding fast in male and female emperor penguins *Aptenodytes forsteri*. Journal of Comparative Physiology B 156: 521-527.

HERZBERG, G.R. (1991). Blood amino acids and the distribution of alanine and aspartate amino transferases in fed and fasted murres (*Uria aalge*). Comparative Biochemistry and Physiology 98A (3-4) : 563-566.

HLADY, D.A. & BURGER, A.E. (1993). Drift-block experiments to analyse the mortality of oiled seabirds off Vancouver Island, British Columbia. Marine Pollution Bulletin 26 (9): 495-501.

HOPE JONES, P., BARRETT, C.F., MUDGE, G.P., HARRIS, M.P. (1984). Physical condition of auks beached in eastern Britain during the wreck of February 1983. Bird Study 31: 95-98.

JAUNIAUX, T., BROSENS, L., MEIRE, P., OFFRINGA, H., COIGNOUL, F. (1998). Pathological investigations on guillemots (*Uria aalge*) stranded at the Belgian coast during the winter 1993-94. Veterinary Record 143: 387-390.

JEFFREY, D.A., PEAKALL, D.B., MILLER, D.S., HERZBERG, G.R. (1985). Blood chemistry changes in food-deprived herring gulls. Comparative Biochemistry and Physiology 81A (4) : 911-913.

JEHL, J.R. Jr. (1997). Cyclical changes in body composition in the annual cycle and migration of the eared grebe (*Podiceps nigricollis*). Journal of Avian Biology 28 : 132-142.

JOIRIS, C.R., TAPIA, G., HOLSBEEK, L. (1997). Increase of organochlorines and mercury levels in common guillemots *Uria aalge* during winter in the southern North Sea. Marine Pollution Bulletin 34 : 1049-1057.

KLASING, K.C. (1997). Nutritional modulation of resistance to infectious diseases. Poultry Science 77 : 119-1125.

LE MAHO, Y., VU VAN KHA, H., KOUBI, H., DEWASMES, G., GIRARD, J., FERRÉ, P., CAGNARD, M. (1981). Body composition, energy expenditure, and plasma metabolites in long-term fasting geese. American Journal of Physiology 241 : E342-E354.

LEMLY, A.D. (1993). Metabolic stress during winter increases the toxicity of selenium to fish. Aquatic Toxicology 27 : 133-158.

LEMLY, A.D. (1996). Winter stress syndrome : an important consideration for hazard assessment of aquatic pollutants. Ecotoxicology and Environmental Safety 34 : 223-227.

NEWMAN, S.H., MAZET, J.K., ZICCARDI, M.H., LIESKE, C.L., FAUQUIER, D.A., GARDNER, I.A., ZINKL, J.G., CHRISTOPHER, M.M. (1999). Haematological changes and anemia associated with captivity and petroleum exposure in seabirds. Comparative Haematology International 9 : 60-67.

NICHOLSON, J.K., KENDALL, M.D., OSBORN, D. (1983). Cadmium and mercury nephrotoxicity. Nature 304 (18): 633-635.

PARSLOW, J.L.F. & JEFFERIES, D.J. (1973). Relationship between organochlorine residues in livers and whole bodies of guillemots. Environmental Pollution 5 : 87-101.

PIATT, J.F. & FORD, R.G. (1996). How many seabirds were killed by the Exxon Valdez oil spill ? American Fisheries Society Symposium 18: 712-719.

PIATT, J.F. & VAN PELT, T.I. (1997). Mass-mortality of Guillemots (*Uria aalge*) in the Gulf of Alaska in 1993. Marine Pollution Bulletin 34 (8):656-662.

ROBIN, J.-P., BOUCONTET, L., CHILLET, P., GROSCOLAS, R. (1998). Behavioral changes in fasting emperor penguins : evidence for a 'refeeding signal' linked to a metabolic shift. American Journal of Physiology 274 (Regulatory Integratice Comparative Physiology 43) : R746-R753.

SANDOVAL, M., HENRY, P.R., LUO, X.G., LITTELL, R.C., MILES, R.D., AMMERMAN, C.B. (1998). Performance and tissue zinc and metallothionein accumulation in chicks fed a high dietary level of zinc. Poultry Science 77 : 1354-1363.

SARTORI, D.R.S., MIGLIORINI, R.H., VEIGA, J.A.S. (1995). Metabolic adaptations induced by long-term starving in quails. Comparative Biochemistry and Physiology 111A (3) : 487-493.

SEISER, P.E., DUFFY, L.K., MCGUIRE, A.D., ROBY, D.D., GOLET, G.H., LITZOW, M.A. (2000). Comparison of pigeon guillemot, *Cepphus columba*, blood parameters from oiled and unoiled areas of Alaska eight years after the *Exxon Valdez* oil spill. Marine Pollution Bulletin 40 (2) : 152-164.

STEWART, F.M. & FURNESS, R.W. (1998). The influence of age on cadmium concentrations in seabirds. Environmental Monitoring and Assessment 50 : 159-171.

TAPIA, G.R. (1998). Mercury and organochlorines in the common guillemot *Uria aalge* wintering in the southern North Sea. Ph.D Thesis, Faculty of Medecine and Pharmacy, Free University of Brussels, Belgium.

THOUZEAU, C., DUCHAMPS, C., HANDRICH, Y. (1999a). Energy metabolism and body temperature of barn owls fasting in the cold. Physiological and Biochemical Zoology 72 : 170-178.

THOUZEAU, C., ROBIN, J.-P., LE MAHO, Y., HANDRICH, Y. (1999b). Body reserve dynamics and energetics of barn owls during fasting in the cold. Journal of Comparative Physiology B 169 : 612-620.

TOTZKE, U., FENSKE, M., HÜPPOP, O., RAABE, H., SCHACH, N. (1999). The influence of fasting on blood and plasma composition of herring gulls (*Larus argentatus*). Physiological and Biochemical Zoology 72(4) : 426-437.

VAN PELT, T.I. & PIATT, J.F. (1995). Deposition and persistence of beachcast seabird carcasses. Marine Pollution Bulletin 30 (12): 794-802.

WENZEL, C. & ADELUNG, D. (1996). The suitability of oiled guillemots (*Uria aalge*) as monitoring organisms for geographical comparisons of trace element contaminants. Archives of Environmental Contamination and Toxicology 31 : 368-377.

Résumé

Résumé

Partie intégrante de l'écosystème marin, les oiseaux de mer sont particulièrement soumis aux pressions exercées sur leur milieu. A fortiori dans une mer aussi fréquentée et exploitée que la Mer du Nord. Bien que reconnue d'importance internationale pour son avifaune, la Mer du Nord n'en reste pas moins un pôle économique stratégique pour ses pays riverains et la cohabitation avec ses non moins importantes ressources naturelles n'est pas sans présenter nombres de difficultés. L'importance d'une gestion durable a néanmoins motivé les différents gouvernements à développer de larges programmes de recherches scientifiques visant à établir un bilan de santé global de la Mer du Nord. C'est dans ce cadre général que les autorités fédérales belges ont notamment lancé, début des années nonante, un programme d'étude des causes de mortalité des oiseaux et des mammifères marins, deux classes de prédateurs au sommet des chaînes trophiques et donc potentiellement indicateurs des niveaux de pollution de l'environnement. Ce travail s'inscrit dans ce schéma général et a pu être réalisée au sein d'un groupe multidisciplinaire réunissant ornithologues, toxicologues et vétérinaires (**M**arine **A**nimal **R**esearch and **In**tervention Network, **MARIN** group).

Cette étude s'est focalisée sur une espèce d'oiseau exclusivement marine, le guillemot de Troïl (*Uria aalge*, Pontopiddan, 1763), dont le séjour à terre se limite à la saison de reproduction quand les individus se rassemblent en colonies. Cette espèce est largement répandue en Mer du Nord, et est plus particulièrement abondante en baie sud pendant la saison d'hivernage, époque pendant laquelle de nombreux individus s'échouent sur les côtes, notamment au littoral belge.

De nombreuses études écotoxicologiques ont considéré des espèces au sommet des réseaux trophiques comme bioindicateurs des niveaux en polluants de l'environnement, et plus spécialement les oiseaux marins. Dans la plupart de ces études ce sont des oiseaux robustes, ne présentant aucun signe de débilitation, qui sont prélevés en mer ou dans leurs colonies.

A contrario, la grande majorité des guillemots échoués à la côte belge sont en très mauvaise condition physique, dans un état d'affaiblissement principalement caractérisé par une fonte plus ou moins importante des muscles pectoraux ainsi que par l'absence de dépôts graisseux sous-cutanés ou abdominaux (état de cachexie). Dans ce contexte d'échouage, il devenait particulièrement intéressant d'examiner l'impact des polluants et plus particulièrement des métaux lourds comme facteurs potentiellement néfastes et débilitants chez des oiseaux déjà soumis à d'autres sources de stress. A cette fin, pour chaque individu examiné, les résultats toxicologiques, pathologiques et écologiques ont été anlysés en parallèle en vue de dégager et clarifier les interactions potentielles entre ces données.

Tous les guillemots examinés durant cette étude (depuis l'hiver 1989-90 jusqu'à l'hiver 1997-98) ont été collectés moribonds ou fraîchement morts à la côte belge pendant la saison d'hivernage (de novembre à début avril). Une première étude (de l'hiver 1989-90 à l'hiver 1993-94), a porté sur les guillemots morts en cours de réhabilitation, et les guillemots directement collectés sur les plages. Une première analyse des résultats démontrera la nécessité de ne plus considérer ces échantillons en commun mais bien de les séparer définitivement. En effet, en plus du peu d'informations disponibles concernant les oiseaux ayant séjourné en centre de réhabilitation, les résultats toxicologiques révèlent d'autre part que ceux-ci présentent des concentrations significativement plus importantes en Zn, Fe, total et inorganique Hg et PCBs dans leurs tissus, que ceux directement collectés sur les plages. Pour ces raisons, à partir de l'hiver 1993-94, les guillemots morts en cours de revalidation ont été automatiquement soustraits de l'échantillonnage et l'effort s'est concentré sur les individus directement prélevés sur les plages. Il n'est pas exclu que quelques individus réhabilités avec succès et relâchés en mer se soient à nouveau échoués et aient donc rejoint le groupe des guillemots collectés directement sur les plages. Néanmoins, le nombre d'individus relâchés avec succès en mer après réhabilitation reste très faible comparé au nombre d'oiseaux 'réellement' échoués pour la même période.

Une grande majorité des guillemots échoués sont retrouvés dans un état de faiblesse extrême, caractérisé à l'autopsie par une fonte plus ou moins importante des muscles pectoraux et une absence quasi totale de graisse sous-cutanée et abdominale (état de cachexie).

Lorsque l'état de cachexie est considéré soit comme 'présent' ou 'absent', les résultats indiquent que les guillemots cachectiques peuvent perdre jusqu'à 32.5 % de leur poids corporel comparés aux individus robustes collectés vivants (± 1 kg).

Si l'on considère par contre différents stades dans la cachexie (depuis non cachectique : '-', à peu '+1', moyennement '+2' et fortement '+3' cachectique), ce qui a été fait à partir de l'hiver 1993-94 , la perte de poids est alors plus prononcée, jusqu'à 43 % chez les individus les plus cachectiques (cachectique '+3', chapitre 3).

Dans ce contexte, l'examen des teneurs en métaux lourds en relation avec cet état de cachexie, (présente ou absente), démontre que les guillemots cachectiques ont des concentrations hépatiques et rénales significativement plus importantes en Cu, Zn, Hg total et inorganique ainsi qu'en PCBs, que les individus les plus robustes de l'échantillonnage (chapitre 2). Ces différences deviennent encore plus flagrantes lorsque les teneurs en métaux lourds chez les oiseaux sont comparées suivant leur état de cachexie : plus les oiseaux sont cachectiques, plus importantes sont les concentrations détectées dans leurs tissus. De plus, l'examen statistique de l'influence potentielle de différentes variables (variables 'explicatives' : sexe, âge, présence ou absence de mazoutage, présence ou absence de **g**astro-**e**ntéropathie **a**ïgue **h**émorrhagique – GEAH -, sévérité de l'état de cahexie) sur les concentrations en métaux lourds (variables 'réponse'), en utilisant une analyse en régression multiple, montre que l'état de cachexie a un effet significativement déterminant sur les concentrations hépatiques, rénales et musculaires (muscle pectoral) en Cu et en Zn. Ces résultats suggèrent qu'une redistribution des métaux lourds comme le Cu et le Zn intervient dans les organes, en réponse à un état d'épuisement prolongé qui provoque et la consommation des lipides de réserves et celle, plus dramatique, des protéines de structure (chapitre 3).

Durant toute cette étude et à l'inverse du Cu et du Zn, les résultats indiquent que les concentrations en Cd dans les tissus dépendent principalement de l'âge des individus et non de la cachexie. Les concentrations sont, de manière générale, significativement plus importantes dans les reins des guillemots adultes comparés aux individus juvéniles. Cependant, il est à noter que les concentrations en Cd détectées chez les guillemots collectés à la côte belge, restent bien inférieures aux concentrations hépatiques et rénales à partir desquelles un empoisonnement au Cd est à craindre.

En complément à la détermination de la sévérité de la cachexie à partir de critères visuels attribués lors de l'autopsie, nous avons également utilisé une valeur quantitative pour estimer l'état général de l'oiseau. Cette valeur est l'indice de condition corporelle obtenu en calculant le rapport du poids frais du foie sur le poids frais du rein, comme proposé par Wenzel & Adelung (1996). Une corrélation avec l'état de cachexie, significativement négative, est démontrée : l'indice étant d'autant moindre que la cachexie est prononcée (chapitre 3).

Comparé aux guillemots collectés dans des régions plus préservées de la Mer du Nord, les résultats indiquent que les guillemots collectés à la côte belge présentent des concentrations plus importantes en Cu, Zn (chapitres 2 et 3) et Hg total (chapitre 2). Une étude spécifique du Hg et des organochlorés (PCBs totaux et p,p'-DDE) chez ces mêmes individus a démontré que des concentrations significativement plus importes de ces contaminants ont été détectées chez les guillemots pendant leur saison d'hivernage en baie sud de la Mer du Nord (Joiris *et al.*, 1997 ; Tapia, 1998). Les auteurs discutent à la fois l'existence d'un cycle saisonnier de contamination/ décontamination et l'existence d'apports, en ces contaminants, plus importants en baie sud de la Mer du Nord. Bien que de telles variations saisonnières n'apparaissent pas en ce qui concerne le Cu, le Zn, le Fe ou le Cd dans notre échantillonnage, les résultats suggèrent également que la baie sud de la Mer du Nord semble être plus contaminée quand on compare les guillemots collectés sur nos côtes et ceux obtenus en Bretagne suite au naufrage du pétrolier Erika (chapitre 4).

Au vu des résultats précédents soulignant l'influence prépondérante de la condition corporelle (état de cachexie) sur les teneurs en métaux lourds des tissus, nous avons donc comparé des guillemots de condition corporelle identique et avons trouvé, invariablement, des teneurs en Cu et Zn significativement plus importantes dans les tissus des guillemots collectés en Belgique. Ce résultat reste entièrement valable même lorsque les individus les plus robustes (indice de condition > 2.5) des deux échantillonnages sont comparés, ce qui tend à suggérer que les niveaux de contaminants sont plus importants en baie sud de la Mer du Nord.

En plus de la comparaison géographique, les résultats obtenus à partir des guillemots collectés en Bretagne nous a permis de confirmer l'existence d'un processus de redistribution général des métaux lourds dans les organes en réponse à l'utilisation des réserves lipidiques et au catabolisme protéique.

Bien que s'agissant de métaux essentiels (Cu et Zn) et vu les concentrations détectées, la question s'est posée de savoir si ces concentrations pouvaient avoir des effets négatifs et agir en tant que facteurs supplémentaires de stress sur la condition corporelle générale des guillemots.

Pour répondre à cette question, des expériences ont été réalisées en utilisant des cailles domestiques (*Coturnix coturnix*) en vue de tester les effets combinés d'une contamination (Cu, Zn et CH_3Hg) et d'un jeûne (chapitre 5). La nourriture distribuée aux cailles a été contaminée de manière à retrouver dans leurs tissus des concentrations similaires à celles détectées chez les guillemots. Suite à cette période de contamination, les cailles furent alors soumises à une période de jeûne complet durant quatre jours. La moitié des cailles qui étaient à la fois contaminées et ayant jeûné 4 jours ont développé un état de cachexie caractéristique, alors que les individus ayant jeûné mais n'ayant pas été contaminés ne présentent aucune des caractéristiques de la cachexie. En outre, il apparaît qu'au sein du groupe contaminé et jeûnant, ce sont les individus cachectiques qui présentent les concentrations en métaux lourds les plus élevés.

Ces résultats suggèrent que, bien que la cachexie liée au jeûne influence nettement les concentrations en métaux lourds détectées dans les organes, ces mêmes concentrations pourraient à leur tour, intervenir dans des processus de débilitation favorisant une baisse généralisée de la condition corporelle des individus.

Le dernier aspect envisagé au cours de cette étude est la spéciation métallique et plus particulièrement les liaisons potentielles pouvant exister entre les métaux lourds et les métallothionéines (MTs). A ce stade de la démarche, il devenait effectivement intéressant d'examiner le rôle joué par les MTs en relation avec la condition corporelle générale des individus. Les résultats indiquent que l'indice de condition des oiseaux influence à la fois la concentration en métal détecté dans les tissus et sa liaison aux MTs, en particulier dans le cas du Cu : plus l'indice de condition est bas, plus la concentration totale en Cu et la concentration de Cu lié aux MTs augmente dans le foie et les reins. D'autre part, dans les deux organes, on remarque que les MTs lient, par µmole de protéine, un pourcentage plus important de Cu et un pourcentage moins important de Zn chez les guillemots les plus faibles (indice de condition $\leqslant 2$). Ces résultats tendent à suggérer que, alors que les concentrations en Cu augmentent chez ces mêmes individus, les ions de Cu supplémentaires se substitueraient au Zn sur la métalloprotéine, ce qui permettrait également au Zn ainsi déplacé de participer à une nouvelle synthèse de MTs. Dans ce contexte, les MTs pourraient être considérées comme une réponse métabolique face à des conditions stressantes, pour les guillemots échoués sur nos côtes, en offrant un mécanisme cytoprotecteur, notamment contre les effets toxiques des ions de Cu.

L'évaluation du rôle des métaux lourds dans une population débilitée de guillemots échoués met en évidence des interactions complexes existant entre les différents contaminants, leurs effets potentiellement néfastes et la condition corporelle générale des individus. Si les concentrations rencontrées en Cu et en Zn ne présentent pas de risques toxicologiques pour des guillemots en bonne condition, il n'en va pas de même pour les sujets chez qui un processus de débilitation est amorcé (indice de condition réduit), les concentrations en ces mêmes éléments pouvant alors influencer négativement le métabolisme général de ces individus affaiblis.

Appendix

English name	*Latin name*
Great auk	*Pinguinus impennis (extinct ± mid 19th century)*
Razorbill	*Alca torda*
Common murre (guillemot)	*Uria aalge*
Thick-billed murre	*Uria lomvia*
Dovekie	*Alle alle*
Black guillemot	*Cepphius grylle*
Pigeon guillemot	*Cepphus columba*
Long-billed murrelet	*Brachyramphus perdix*
Marbeld murrelet	*Brachyramphus marmoratus*
Kittlitz's murrelet	*Brachyramphus brevirostris*
Xantu's murrelet	*Endomychura hypoleuca* or *Synthliboramphus hypoleucus*
Craveri's murrelet	*Endomychura craveri* or *Synthliboramphus craveri*
Ancient murrelet	*Synthliboramphus antiquus*
Japonese murrelet	*Synthliboramphus wumizusume*
Whiskered auklet	*Aethia pygmaea*
Least auklet	*Aethia pusilla*
Crested auklet	*Aethia cristatella*
Cassin's auklet	*Ptychoramphus aleuticus*
Parakeet auklet	*Cyclorrhynchus psittacula*
Atlantic puffin	*Fratercula arctica*
Horned puffin	*Fratercula corniculata*
Tufted puffin	*Lunda cirrhata* or *Fratercula cirrhata*
Rhinoceros auklet	*Cerorhinca monocerata*

Table 1 : list of the 22 species of the Alcidae family - Vertebrata, Aves, Charadriiformes, Alcidae – as proposed by Storer (1964), presented in Bédart, 1985 (see list of references in chapter 1).

Biota	Species	Tissues	Location	Cu	Zn	Cd	Pb	Hg	Reference
Seawater	Dissolved metals		East coast of Britain	0.12 - 0.58	nd	0.01 – 0.06	0.02 – 0.14	nd	Balls, 1985a.
Seawater	Dissolved metals		Central North Sea	0.06 – 0.23	nd	0.006 – 0.02	0.02 – 0.04	nd	Balls, 1985b.
Seawater	Dissolved metals		Central North Sea	0.23 – 0.39	0.16 – 0.61	0.02 – 0.03	0.017 – 0.032	0.0002 – 0.0004	Fileman *et al.*, 1991.
Seawater	Dissolved metals		Subregion 4: Dutch and Belgian coasts	0.3 - 1.0	nd	0.02 - 0.05	0.03 - 0.12	nd	North Sea Task Force, 1993a.
Seawater	Dissolved metals		Subregion 7a+b:Central North Sea	0.10 - 0.47	0.16 - 1.4	0.006 - 0.09	0.017 - 0.022	0.005 - 0.008	North Sea Task Force, 1993c,d.
Seawater	Dissolved metals		Subregion 1: Northern North Sea	0.02- 0.41	nd	0.004 - 0.016	0.02 – 0.1	0.001 – 0.003	North Sea Task Force, 1993e.
Seawater	Dissolved metals		Southern North Sea < 53° N	0.29 – 0.57	0.22 – 1.4	0.004 – 0.022	0.024 – 0.041	0.0001 – 0.002	Law *et al.*, 1994.

Seawater	Dissolved metals		Central North Sea > 53° N	0.31 – 0.56	0.27 – 0.55	0.01 – 0.05	0.03 – 0.08	0.002 – 0.004	Law *et al.*, 1994.
Seawater	Dissolved metals		Central North Sea > 55° N	nd	nd	0.013 ± 0.001	0.054 ± 0.03	nd	Scholten *et al.*, 1998.
Seawater	Dissolved metals		Southern Bight	0.33 ± 0.02	1.15 ± 0.11	0.032 ± 0.002	0.152 ± 0.019	nd	Scholten *et al.*, 1998.
Seawater	Dissolved metals		Dutch coast	0.74 ± 0.03	1.74 ± 0.09	0.053 ± 0.002	0.117 ± 0.012	nd	Scholten *et al.*, 1998.
Seawater	Particulate metals		Central North Sea > 53° N	1.3 – 5.3	3.2 - 31	0.13 – 1.4	0.69 – 3.4	0.04 – 0.21	Fileman *et al.*, 1991.
Seawater	Particulate metals		North Sea	18 –43	73 - 1100	0.10 – 1.4	16 - 88	nd	Laslett, 1995.
Seawater	Particulate metals		English Channel	13 - 18	76 - 320	0.26 – 0.71	17 - 35	nd	Laslett, 1995.
Invertebrates	Mussel *Mytilus edulis*	Soft tissue	Belgian coast	1.2	25.3	0.17	0.31	0.01	Gobert *et al.*, 1992.
Invertebrates	Mussel *Mytilus edulis*	Soft tissue	Orkney Islands 59°N – 58°N	0.7	9.4	0.13	0.51	0.01	Brown & Balls, 1997.

Invertebrates	Scallop *Pecten maximus*	Soft tissue	Northeast Scotland 58°N – 56°N	0.5	23.6	0.18	0.14	0.01	Brown & Balls, 1997.
Invertebrates	Starfish *Asteria rubens*	Soft tissue	Belgian coastal waters	2.1 ± 0.9 0.8 - 5.2	43 ± 13.0 20 - 72	0.10 ± 0.05 0.04 - 0.21	0.28 ± 0.17 0.07 - 0.86	0.05 ± 0.02 0.02 - 0.10	Guns *et al.*, 1999.
Invertebrates	Hermit crab *Pagurus bernhardus*	Soft tissue	Belgian coastal waters	29 ± 6.6 18 - 43	31 ± 5.9 25 - 49	0.07 ± 0.03 0.03 - 0.15	0.28 ± 0.16 0.03 - 0.56	0.03 ± 0.01 0.02 - 0.06	Guns *et al.*, 1999.
Invertebrates	Brown shrimp *Crangon crangon*	Soft tissue	Belgian coastal waters	8.7 ± 2.9 5.4 - 20.0	29 ± 4.6 21 - 46	0.03 ± 0.02 0.01 - 0.08	0.19 ± 0.15 0.03 - 0.68	0.05 ± 0.01 0.03 - 0.07	Guns *et al.*, 1999.
Fish	Cod *Gadus morhua*	Muscle	Central North Sea 57°N - 54°N	0.13	3.6	< 0.001	< 0.02	0.18	Brown & Balls, 1997.
		Muscle	Northeast Scotland 58°N – 56°N	0.12	3.8	< 0.001	< 0.02.	0.17	Brown & Balls, 1997.

	Muscle	Orkney and Shetland Islands	0.10	3.9	< 0.001	< 0.02	0.04	Brown & Balls, 1997.
Haddock *Melanogrammus aeglefinus*	Muscle	Central North Sea 57°N - 54°N	0.09	3.0	< 0.001	< 0.02	0.05	Brown & Balls, 1997.
	Muscle	Northeast Scotland	0.10	3.5	< 0.001	< 0.02	0.05	Brown & Balls, 1997.
	Muscle	Orkney and Shetland Islands	0.20	4.0	< 0.001	< 0.02	0.05	Brown & Balls, 1997.
	Muscle	Norwegian coasts	0.12	3.3	< 0.001	< 0.02	0.06	Brown & Balls, 1997.
Saithe *Pollachius virens*	Muscle	Central North Sea 57°N - 54°N	0.60	4.5	< 0.001	< 0.02	0.10	Brown & Balls, 1997.
	Muscle	Sheltand Islands	0.34	5.5	< 0.001	< 0.02	0.03	Brown & Balls, 1997.
	Muscle	Norwegian coasts	0.30	5.7	< 0.001	< 0.02	0.05	Brown & Balls, 1997.
Whiting *Merlangius merlangus*	Liver	Subregion 3b: East coast of England	4.02 1.7 – 5.3	17.4 13 - 23	0.11 < 0.06 – 0.23	< 0.70	0.04 0.02 – 0.07	North Sea Task Force, 1993b.

Whiting *Merlangius merlangus*	Muscle	Subregion 3b: East coast of England	0.21 0.09 – 0.35	3.1 2.7 – 3.7	nd	nd	0.08 0.03 – 0.14	North Sea Task Force, 1993b.
Whiting *Merlangius merlangus*	Muscle	Central North Sea 57°N - 54°N	0.13	3.8	< 0.001	< 0.02	0.06	Brown & Balls, 1997.
	Muscle	Northeast Scotland 58°N – 56°N	0.17	3.5	< 0.001	< 0.02	0.04.	Brown & Balls, 1997.
	Muscle	Shetland Islands	0.18	3.9	< 0.001	< 0.02	0.04	Brown & Balls, 1997.
	Muscle	Norwegian coasts	0.18	3.9	< 0.001	< 0.02	0.03	Brown & Balls, 1997.
Flounder *Platichthys flesus*	Liver	The Great Belt Kattegat	15.0	45.0	0.14	1.2	nd	Jørgensen & Pedersen, 1994.
Plaice *Pleuronectes platessa*	Liver	Skagerrak Danish coasts	2.5	30.0	0.09	1.6	nd	Jørgensen & Pedersen, 1994.

Table 2: trace elements concentration in different compartments of the North Sea.

- expressed as a range (min-max concentrations) in µg/l for dissolved metals;
- expressed as a mean ± standard error µg/l in Scholten *et al.*, 1998;
- and in µg/g d.w. for metals in suspended particulate matter;
- expressed as a mean (+ min – max values when available) in µg/g w.w. for invertebrates and fishes.

Table 2 : references

BROWN & BALLS (1997). Trace metals in fish and shellfish from Scottish waters. Scottish Fisheries Research Report 60 : 2-37.

BALLS, P.W. (1985a). Copper, lead and cadmium in coastal waters of the western North Sea. Marine Chemistry 15 : 363-378.

BALLS, P.W. (1985b). Trace metals in the northern North Sea. Marine Pollution Bulletin 16 (5) : 203-207.

FILEMAN, C. F., ALTHAUS, M., LAW, R.J., HASLAM, I. (1991). Dissolved and particulate trace metals in surface waters over the Dogger Bank, central North Sea. Marine Pollution Bulletin 22 (5) : 241-244.

GOBERT, S., DAEMERS-LAMBERT, C., BOUQUEGNEAU, J.-M. (1992). Etat physiologique et contamination par les métaux lourds des moules le long du littoral belge. Bulletin de la Société Royale des Sciences de Liège 61 (1-2) : 177-194.

GUNS, M., VAN HOEYWEGHEN, P., VYNCKE, W., HILLEWAERT, H. (1999). Trace metals in selected benthic invertebrates from Belgian coastal waters (1981-1996). Marine Pollution Bulletin 38 (12) : 1184-1193.

JØRGENSEN, L.A. & PEDERSEN, B. (1994). Trace metals in fish used for time trend analysis and as environmental indicators. Marine Pollution Bulletin 28 (1): 24-32.

LASLETT, R.E. (1995). Concentrations of dissolved and suspended particulate Cd, Cu, Mn, Ni, Pb and Zn in surface waters around the coasts of England and Wales and in adjacent seas. Estuarine, Coastal and Shelf Science 40 : 67-85.

LAW, R.J., WALDOCK, M.J., ALLCHIN, C.R., LASLETT, R.E., BAILEY, K.J. (1994). Contaminants in seawater around England and Wales : results from monitoring surveys, 1990-1992. Marine Pollution Bulletin 28 (11) : 668-675.

NORTH SEA TASK FORCE REPORT (1993a). North Sea Subregion 4: assessment report (Netherlands, Belgium, France, Germany and United Kingdom). Oslo and Paris Commission (Olsen & Olsen, Eds), 195 pp.

NORTH SEA TASK FORCE REPORT (1993b). North Sea Subregion 3b: assessment report: United Kingdom. Oslo and Paris Commission (Olsen & Olsen, Eds), 154 pp.

NORTH SEA TASK FORCE REPORT (1993c). North Sea subregion 7a : United Kingdom, Belgium, Denmark, Germany, Netherlands. Oslo and Paris Commission (Olsen & Olsen, Eds), 86 pp.

NORTH SEA TASK FORCE REPORT (1993c). North Sea subregion 7b: United Kingdom, Belgium, Denmark, Germany, Netherlands. Oslo and Paris Commission (Olsen & Olsen, Eds), 140 pp.

NORTH SEA TASK FORCE REPORT (1993e). North Sea subregion 1 : Norway, United Kingdom. Oslo and Paris Commission (Olsen & Olsen, Eds), 44 pp.

SCHOLTEN, M.C.TH., KRAMER, K.J.M., LAANE, R.W.P. (1998). Trends and variation in concentration of dissolved metals (Cd, Cu, Pb, and Zn) in the North Sea (1980 – 1989). ICES Journal of Marine Science 55 : 825-834.

Age status	Ringing place	Ringing coord.	Finding place	Finding coord.	Finding details
< 2 years	Handa Island, Scourie, Scottish Highlands 04.06.1972	58° 23 N 05° 11 W	Blankenberge West Vlaandereen 30.12.1980	51° 19 N 03° 08 E	oiled and dying.
pullus	Foula Shetland Scotland 25.06.1981	60° 08 N 02° 05 W	Lombardsijde West Vlaanderen 29.01.1982	51° 09 N 02° 45 E	found dead.
pullus	Isle of May Fife region Scotland 20.06.1993	56° 11 N 02° 34 W	Nieuwpoort West Vlaanderen 31.01.1994	51° 08 N 02° 45 E	found freshly dead and oiled.
pullus	Isle of May Fife region Scotland 10.06.1992	56° 11 N 02° 34 W	Nieuwpoort West Vlaanderen 30.12.1993	51° 08 N 02° 45 E	found oiled: released 10.01.1994
pullus	Isle of May Fife region Scotland 14.06.1992	56° 11 N 02° 34 W	Nieuwpoort West Vlaanderen 30.12.1993	51° 08 N 02° 45 E	found freshly dead.
pullus	Whynnifold Grampian region Scotland 20.06.1987	57° 23 N 02° 55 E	Oostende West Vlaanderen 14.02.1990	51° 17 N 03° 05 E	found freshly dead and oiled.
pullus	Badbea, Helmsdale Scotland 20.06.1990	58° 09 N 03° 33 W	Antwerpen 14.12.1990	51° 13N 04° 25 E	found dead (oiled).
pullus	North Berwick Lothian region Scotland 29.06.1975	56° 04 N 02° 45 W	Blankenberge West Vlaandereen 02.03.1990	51° 19 N 03° 08 E	found dead (oiled).
pullus	An Dun, Dunbeath Highland, Scotland 24.06.1987	58° 13 N 03° 28 W	De Panne West Vlaanderen 04.03.1990	51° 06 N 02° 35 E	found freshly dead and oiled.
pullus	Isle of May Fife region Scotland 20.06.1984	56° 11 N 02° 34 W	Westende West Vlaanderen 00.02.1985	51° 10 N 02° 46E	found freshly dead.
pullus	Whynnifold Grampian region Scotland 30.06.1979	57° 23 N 02° 55 E	Nieuwpoort West Vlaanderen 03.01.1986	51° 08 N 02° 45 E	found freshly dead.

pullus	Isle of May Fife region Scotland 19.06.1987	56° 11 N 02° 34 W	Zeebrugge West Vlaanderen 06.01.1988	51° 20 N 03° 12 E	found freshly dead and oiled. *Uria aalge albionis*
pullus	Whynnifold Grampian region Scotland 30.06.1979	57° 23 N 02° 55 E	Blankenberge West Vlaandereen 16.02.1988	51° 19 N 03° 08 E	found dead (oiled).
pullus	Badbea, Helmsdale Scotland 22.06.1988	58° 09 N 03° 33 W	Wenduine West Vlaanderen 10.12.1988	51° 18 N 03° 05 E	found dead.
pullus	North Sutor, Nigg Highland Scotland 25.06.1982	57° 42 N 04° 00 W	Nieuwpoort West Vlaanderen 01.01.1984	51° 08 N 02° 45 E	found freshly dead and oiled. *Uria aalge albionis*
pullus	Isle of May Fife region Scotland 26.06.1994	56° 11 N 02° 34 W	Lombardsijde West Vlaanderen 04.02.1995	51° 09 N 02° 45 E	found freshly dead. *Uria aalge* full grown.
pullus	Whynnifold Grampian region Scotland 24.06.1978	57° 23 N 02° 55 E	Nieuwpoort West Vlaanderen 00.01.1985	51° 08 N 02° 45 E	found freshly dead. *Uria aalge* > 1 year.
pullus	Helmsdale, Highland Scotland 19.06.1990	58° 09 N 03° 34 W	Wenduine West Vlaanderen 01.01.1995	51° 18 N 03° 05 E	found freshly dead.
> 1 year	Porlock Weir/ Minehead, Somerset England 08.02.1990	51° 13 N 03° 37 W	De Haan West Vlaanderen 12.02.1990	51° 16 N 03° 02 E	found freshly dead and oiled.
pullus	Skomer Island Wales England 03.07.1989	51° 44 N 05° 19 W	De Haan West Vlaanderen 09.02.1990	51° 16 N 03° 02 E	found dead.
pullus	Great Saltee, Wexford England 23.06.1987	52° 07 N 06° 38 W	De Haan West Vlaanderen 03.01.1988	51° 16 N 03° 02 E	found freshly dead and oiled. *Uria aalge albionis*
pullus	Great Saltee, Wexford England 23.06.1986	52° 07 N 06° 38 W	Nieuwpoort West Vlaanderen 28.03.1988	51° 08 N 02° 45 E	found dead (oiled).

pullus	Great Saltee, Wexford England 22.06.1992	52° 07 N 06° 38 W	Zeebrugge West Vlaanderen 03.01.1995	51° 20 N 03° 12 E	found dead (oiled).
> 1 year	Westbank Ter Hoogte Westkapelle Zeeland The Netherlands 14.04.1994	51° 34 N 03° 24 E	Nieuwpoort West Vlaanderen 20.04.1994	51° 08 N 02° 45 E	found dying.
> 1 year	Waddenzee Ameland The Netherlands 06.02.1990	53° 27 N 05° 36 E	Middlekerke West Vlaanderen 18.02.1990	51° 11 N 02° 49 E	found freshly dead.
pullus	Helgoland Schleswig Holstein Germany 18.06.1991	54° 11 N 07° 55 E	Zeebrugge West Vlaanderen 21.12.1991	51° 20 N 03° 12 E	found dead (oiled).
> 1 year	Helgoland Schleswig Holstein Germany 13.08.1986	54° 11 N 07° 55 E	De Panne West Vlaanderen 07.02.1988	51° 06 N 02° 35 E	found dead (oiled).
pullus	Stora Karlsö Gotland Sweden 06.07.1972	57° 17 N 17° 58 E	Oostende West Vlaanderen 12.01.1974	51° 17 N 03° 05 E	found freshly dead.

Table 3: list of identified ringed guillemots stranded at the Belgian coast over the last 25 years. Information kindly communicated by W. Rogemman and J. Tavernier, **R**oyal **I**nstitute of **N**atural **S**ciences of **B**elgium (IRSNB, Brussels).

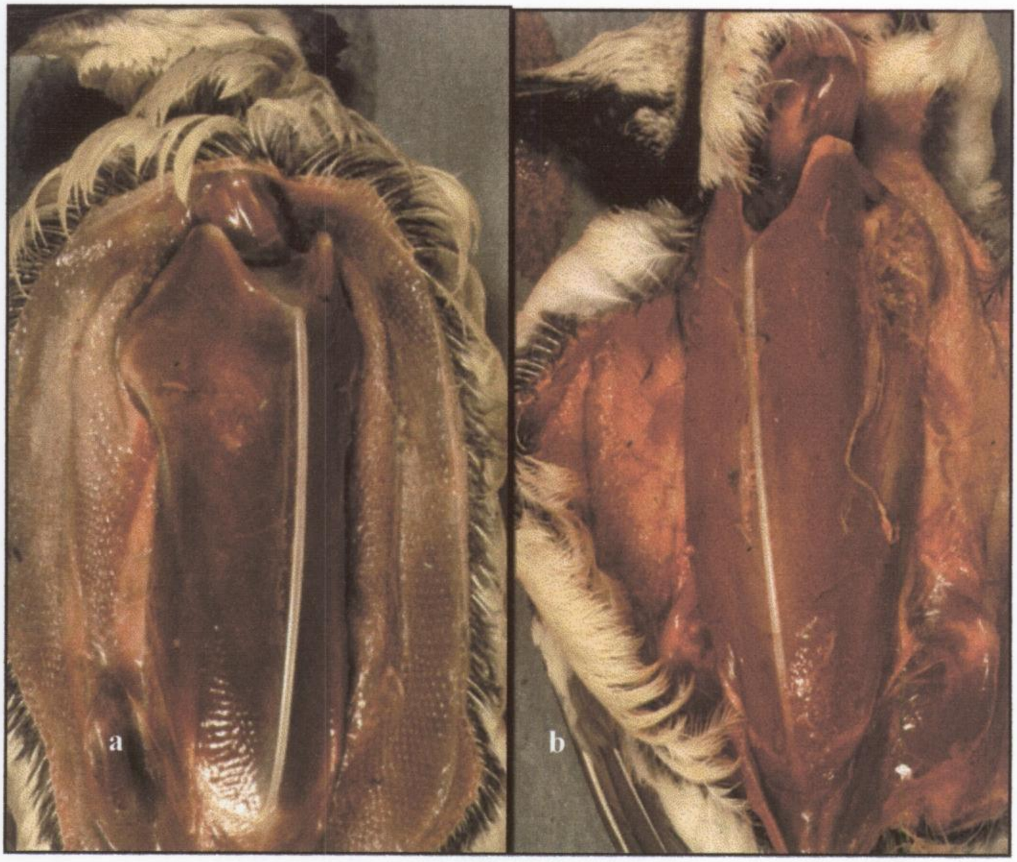


Photo 1 a : abnormally atrophiated pectoral muscle of a cachectic guillemot.
b : pectoral muscle of a non cachectic, robust guillemot.
Photos kindly received from Dr. T. Jauniaux.

General information

English name	***Latin name***	**French name**
Lesser sandeel	*Ammodytes sp*	Lançon
Herring	*Clupea harengus*	Hareng
Cod	*Gadus morhua*	Morue, cabillaud
Capelin	*Mallotus villosus*	Capelan
Haddock	*Melanogrammus aegelfinus*	Eglefin
Whiting	*Merlangius merlangus*	Merlan
Saithe	*Pollachius virens*	Lieu noir
Sprat	*Sprattus sprattus*	Sprat
Norway pout	*Trisopterus esmarkii*	Tacaud norvégien
Flounder	*Platichthys flesus*	Flet
Plaice	*Pleuronectes platessa*	Plie, carrelet
Mussel	*Mytilus edulis*	Moule
Brown shrimp	*Crangon crangon*	Crevette grise
Scallop	*Pecten maximus*	Coquille Saint-Jacques
Common limpet	*Patella vulgata*	Patelle
Starfish	*Asteria rubens*	Etoile de mer
Hermit crab	*Pagurus bernhardus*	Bernard L'Hermite
The Great Penguin (extinct species)	*Pinguinus impennis*	Le Grand Pingouin (espèce éteinte)
King Penguin	*Aptenodytes patagonicus*	Manchot empereur
Adelie penguin	*Pygoscelis adeliae*	Manchot Adélie
Puffin	*Fratercula arctica*	Macareux moine
Razorbill	*Alca torda*	Petit pingouin
Rhinoceros auklet	*Cerorhinca monocerata*	Macareux huppé
Kittiwake	*Rissa tridactyla*	Mouette rieuse
Petrel	*Fulmarus glacialis*	Pétrel fulmar
Giant petrel	*Macronectes halli*	Fulmar de Hall
Manx shearwater	*Puffinus puffinus*	Puffin
Short-tailed shearwater	*Puffinus tenuirostris*	Puffin à queue courte
Cory's shearwater	*Calonectris diomedea*	Puffin cendré
Eider	*Somateria mollissima*	Eider à duvet
Spectacled eider	*Somateria fischeri*	Eider à lunettes
Great northern diver (common loon, USA)	*Gavia immer*	Plongeon imbrin
Oystercatcher	*Haematopus ostralegus*	Huitrier-pie
Great skua	*Catharacta skua*	Grand labbe
Lesser black-backed gull	*Larus fuscus*	Goéland brun
Eared grebe	*Podiceps nigricollis*	Grèbe à cou noir
Flamingo	*Phaenicopterus ruber*	Flamant rose
Little egret	*Egretta garzetta*	Aigrette garzette
Mallard duck	*Anas platyrhynchos*	Canard colvert
Starling	*Sturnus vulgaris*	Etourneau
Barn owl	*Tyto alba*	Chouette effraie

Table a : list of Latin, English and French names used in this study.

Glossary: Academic Press Dictionary of Science and Technology (http://www.harcourt.com/dictionary/).

Alcohol dehydrogenase: *Enzymology*. Enzymes catalyzing the oxidation of ethanol, vitamin A alcohol, and certain sterols, using NAD as cofactor. Found in microorganisms and higher organisms.

Carbonic anhydrase: *Enzymology*. An enzyme involved in the transport and release of carbon dioxide by acting on carbonic acid to produce CO_2 in physiological processes; it occurs primarily in the blood and in the mucous membrane of the stomach lining. Also, carbonate dehydratase, carbonic acid anhydrase, carboanhydrase.

Carboxypeptidase: *Enzymology*. A class of enzymes catalyzing the hydrolysis of amino acid residues from the carboxyl terminal portion of the peptide chains.

Cytokine: *Immunology*. The soluble molecules being produced by cells that mediate reactions between cells, usually used for biological response modifiers.

Demersal: *Ecology*. Found in deep water or in the bottom of streams, pools, or the ocean: said specially of certain fish eggs.

Dredging: *Engineering*. the process of excavating solid matter from an underwater area.

Emetic: *Pharmacology*. Any agent that induces vomiting.

Fledging: *Ecology*. Period at which the young leaves the colony.

Glutathione: *Biochemistry*. $C_{10}H_{17}O_6N_3S$, a naturally occurring peptide, serving as a biological redox agent, a coenzyme, or a cofactor or substrate for certain coupling reactions catalyzed by ligandin; an important substance in tissue oxidation.

Pelagic: *Oceanography*. Of or relating to the open ocean, near the surface or in the middle depths, beyond the littoral zone and above the abyssal zone;
Hydrology. Relating to the deeper regions of a lake that are characterized by deposits of mud or ooze and by the absence of aquatic vegetation ;
Ecology. Of or relating to aquatic organisms that live in the ocean, without direct dependence on the shore or bottom or on deep-sea sediment. (Going back to a Greek word meaning "the sea.").

Prognosis: *Medicine*. a prediction of the probable course or outcome of a disease, injury, or developmental abnormality, based on such factors as the patient's condition and medical history and the usual course of the disease or abnormality.

Pullus: *Ecology*. Bird ringed as a chick at the colony.

Rearing: *Ecology*. The raising of young by the parents.

Spawning season:. *Zoology*. The season at which the fishes, amphibians, mollusks, crustaceans, and the like lay their eggs or sperm directly into the water.

Stenophagous: *Ecology*. Describing an organism that feeds on a narrow range of species. Also, stenophagic.

Superoxide dismutase: *Enzymology*. An enzyme that catalyzes the reaction of two molecules of the potentially harmful compound superoxide to form oxygen and hydrogen peroxide.

Transferrin: *Hematology*. Serum -globulin that binds and transports iron. Several types (C, B, D, and many others) have been distinguished on the basis of electrophoretic mobility and related as the products of corresponding dominant somatic genes, Tf^C, Tf^B, and Tf^D.

Unsaturated fatty acid: *Nutrition*. An organic compound of carbon, hydrogen, and oxygen that combines with glycerol to form fat; it contains one (mono-, *i.e.:* oleic acid) or more (poly-, *i.e.:* linoleic 18:2 n-6, arachidonic 20:4 n-6, eicosapentaenoic 20:5 n-3 acids) double bonds and is liquid at room temperature.

Colony / Geographical location	Authors / Year of publication	Reference
Latrabjerg, Northwest Iceland.	Barrett, R.T., Peterz, M., Furness, R.W., Durinck, J. (1989). The variability of biometric measurements.	Ring. Migr. 10: 13-16.
Stora Karlsö (Baltic Sea).	Hedgren, S. & Linnman, A. (1979). Growth of guillemot *Uria aalge* chicks in relation to time of hatching.	Ornis Scandinavia 10: 29-36.
Skagerrak (Baltic Sea).	Blake, B.F. (1983). A comparative study of the diet of auks killed during an oil accident in the Skagerrak in January 1981.	J. Zool. Lond. 201: 1-12.
Persfjord and Bøkfjord, eastern Finmark.	Erikstad, K.E. & Vader, W. (1989). Capelin selection by common and Brunnïch's guillemots during the prelaying season.	Ornis Scandinavia 20: 151-155.
Hornøy, Norway.	Furness, R.W. & Barrett, R.T. (1985). The food requirements and ecological relationships of a seabird community in North Norway.	Ornis Scandinavia 16: 305-313.
Hornøy, Norway.	Barrett, R.T. & Furness, R.W. (1990). The prey and diving depth of seabirds on Hornøy, North Norway after a decrease in the Barents Sea capelin stocks.	Ornis Scandinavia 21: 179-186.
Hornøy, Bleiksøy, Runde, Bjørnøy, Norway.	Moum, T., Erikstad, K.E., Bjørklid, E. (1991). Restriction fragment analysis of mitochondrial DNA in common murres, *Uria aalge*, from four Norwegian seabird colonies.	Can. J. Zool. 69: 1577-1584.
Hornøya, Norway.	Wenzel, C. & Gabrielsen, G.W. (1995). Trace element accumulation in three seabird species from Hornøya, Norway.	Arch. Environ. Contam. Toxicol. 29: 198-205.
Hornøya, Norway.	Gabrielsen, G.W. (1996). Energy expenditure of breeding common murres.	Occasional Paper of the Canadian Wildlife Service, 91: 49-58.
Hornøya, Norway. Brittany, France.	Wenzel, C. & Adelung, D. (1996). The suitability of oiled guillemots (*Uria aalge*) as monitoring organisms for geographical comparisons of trace element contaminants.	Arch. Environ. Contam. Toxicol. 31: 368-377.
Østfold County, Norway	Lorentsen, S.-H. & Anker-Nielsen, T. (1999). Diet of common murres wintering in the northern Skagerrak during 1988-1990: variation with sex, age and season.	Waterbirds 22 (1): 80-89.
Barents Sea.	Barrett, R.T. & Krasnov, Y.V. (1996). Recent responses to changes in stock of prey species by seabirds breeding in the southern Barents Sea.	Ices Journal of Marine Science 53: 713-722.

Bogoslof Island, Bering Sea.	Iversen, J.A. & Krog, J. (1972). Body temperatures in active and resting Charadriiforme birds (murres, puffins and auklets) at different ambient temperatures.	Norw. J Zool. 20: 145-146.
Greenland, North Atlantic.	Birkhead, T.R. (1986). Counts of bridled guillemots *Uria aalge* from Jan Mayen and southwest Greenland.	Ornis Scandinavia 17: 363-364.
Central North Sea (ship-based surveys)	Camphuysen, C.J. (1998). Diurnal activity patterns and nocturnal group formation of wintering common murres in the Central North Sea.	Colonial Waterbirds 21 (3) : 406-413.
Skagerrak, Kattegat (ship-based surveys)	Skov, H., Durinck, J., Andell, P. (2000). Associations between wintering avian predators and schooling fish in the Skagerrak-Kattegat suggest reliance on predictable aggregations of herring *Clupea harengus*.	Journal of Avian Biology 31 : 135-143.
Helgoland, North Sea.	Vauk, G., Prüter, J., Hartwig, E (1989). Long-term population dynamics of breeding bird species in the German Wadden Sea area.	Helgoländer Meeresunters 43: 357-365.
Helgoland, North Sea.	Leopold, , M.F., Wolf, P.A., Hüppop, O. (1992). Food of young and colony attendance of adult guillemot *Uria aalge* on Helgoland.	Helgoländer Meeresunters 46: 237-249.
Skomer Island, Wales, England.	Birkhead, T.R. (1974). Movement and mortality rates of British Guillemots.	Bird Study 21: 241-254.
Skomer Island, Wales, England.	Birkhead, T.R. (1977). Adaptative significance of the nestling period of guillemot *Uria aalge*.	Ibis 119: 544-549.
Skomer Island, Wales, England.	Birkhead, T.R. & Taylor, A.M. (1977). Moult of the guillemot *Uria aalge*.	Ibis 119: 80-85.
Skomer Island, Wales, England.	Birkhead, T.R. (1977). The effect of habitat and density on breeding success in the common guillemot (*Uria aalge*).	J. Anim. Ecol. 46: 751-764.
Skomer Island, Wales, England.	Hatchwell, B.J. (1991). The feeding ecology of young guillemots *Uria aalge* on Skomer Island, Wales.	Ibis 133: 153-161.
Skomer Island, Wales, England.	Hatchwell, B.J. (1991). An experimental study of the effects of timing of breeding on the reproductive success of common guillemots (*Uria aalge*).	J. Anim. Ecol. 60: 721-736.
Skomer Island, Wales, England.	Hatchwell, B.J. & Birkhead, T.R. (1991). Population dynamics of common guillemots *Uria aalge* on Skomer Island, Wales.	Ornis Scandinavia 22: 55-59.

Skomer Island, Wales, England.	Parker, J.S., Birkhead, T.R., Joshua, S.K., Taylor, S., Clark, M.S. (1991). Sex ratio in a population of guillemots *Uria aalge* determined by chromosome analysis.	Ibis 133: 423-426
Fair Isle, England.	Birkhead, T.R. (1986). Feeding ecology of common guillemots on Fair Isle, 1985.	Bull. Br. Ecol. Soc. 17 (1): 13-15.
Canna Inner Hebrides, Scotland.	Swann, R.L. & Ramsay, D.K. (1983). Movements from and age of return to an expanding Scottish guillemot colony.	Bird Study 30: 207-214.
Canna Inner Hebrides, Scotland.	Harris, M.P., Halley, D.J., Swann, R.L. (1994). Age of first breeding in common murres.	Auk 111 (1): 207-209.
North and East Scotland.	Blake, B.F., Dixon, T.J., Jones, H.P., Tasker, M.L. (1985). Seasonal changes in the feeding ecology of guillemots (*Uria aalge*) off North and East Scotland.	Estuarine, Coastal and Shelf Science 20: 559-568.
Cruden Bay, North and East Scotland.	Murray, S., Wanless, S., Harris, M.P. (1994). The effects of fixed salmon Salmo salar nets on the guillemot *Uria aalge* and razorbill *Alca torda* in Northeast Scotland in 1992.	Biological Conservation 70: 251-256.
Summer Isles, north-west Scotland.	Furness, R.W., Thompson, D.R., Harrison, N. (1994). Biometrics and seasonal changes in body composition of common guillemots *Uria aalge* from north-west Scotland.	Seabird 16: 22-29.
Pembrokeshire, Farne Islands.	Harris, M.P. (1970). Differences in the diet of British Auks.	Ibis 112: 540-541.
Isle of May, Firth of Forth, Scotland.	Harris, M.P. & Wanless, S. (1984). The effect of the wreck of seabirds in February 1983 on auk populations on the Isle of May.	Bird Study 31: 103-110.
Isle of May, Firth of Forth, Scotland.	Harris, M.P. & Wanless, S. (1985). Fish fed to young guillemots, *Uria aalge*, and used in dispaly on the Isle of May, Scotland.	J. Zool., Lond. 207: 441-458.
Isle of May, Firth of Forth, Scotland.	Harris, M.P. & Wanless, S. (1986). The food of young razorbills on the Isle of May and a comparison with that of guillemots and puffins.	Ornis Scandinavia 17: 41-46.
Isle of May, Firth of Forth, Scotland.	Harris, M.P. & Wanless, S. (1988). Measurements and seasonal changes in weight of guillemots *Uria aalge* at a breeding colony.	Ring. Migr. 9: 32-36.
Isle of May, Firth of Forth, Scotland.	Harris, M.P. & Wanless, S. (1988). The breeding biology of guillemots *Uria aalge* on the Isle of May over a six year period.	Ibis 130: 172-192.

Isle of May, Firth of Forth, Scotland.	Wanless, S., Morris, J.A., Harris, M.P. (1988). Diving behaviour of guillemot *Uria aalge*, puffin, *Fratercula arctica* and razorbill *Alca torda* as shown by radio-telemetry.	J. Zool., Lond. 216: 73-81.
Isle of May, Firth of Forth, Scotland.	Harris, M.P. (1989). Variation in the correction factor used for converting counts of individual guillemots *Uria aalge* into breeding pairs.	Ibis 131: 85-93
Isle of May, Firth of Forth, Scotland.	Harris, M.P. & Wanless, S. (1990). Moult and autumn colony attendance of auks.	Brit. Birds 83: 55-67.
Isle of May, Firth of Forth, Scotland.	Harris, M.P. & Bailey, R.S. (1992). Mortality rates of puffin *Fratercula arctica* and guillemot *Uria aalge* and fish abundance in the North Sea.	Biol. Cons. 60: 39-46.
Isle of May, Firth of Forth, Scotland.	Harris, M.P., Halley, D.J., Wanless, S. (1992). The post fledging survival of young guillemots *Uria aalge* in relation to hatching date and growth.	Ibis 134: 335-339.
Isle of May, Firth of Forth, Scotland.	Halley, D.J. & Harris, M.P. (1993). Intercolony movement and behaviour of immature guillemots *Uria aalge*.	Ibis 135: 264-270.
Isle of May, Firth of Forth, Scotland.	Harris, M.P., Halley, D.J., Swann, R.L. (1994). Age of first breeding in common murres.	Auk 111 (1): 207-209.
Isle of May, Firth of Forth, Scotland.	Halley, D.J., Harris, M.P., Wanless, S. (1995). Colony attendance patterns and recruitement in immature common murres (*Uria aalge*).	Auk 112: 947-957.
Isle of May, Firth of Forth, Scotland.	Harris, M.P. & Wanless, S. (1995). Survival and non-breeding of adult common guillemot *Uria aalge*.	Ibis 137: 192-197.
Isle of May, Firth of Forth, Scotland.	Harris, M.P. & Wanless, S. (1996). Differential responses of common guillemot *Uria aalge* and shag *Phalacrocorax aristotelis* to a late winter wreck.	Bird Study 43: 220-230.
Isle of May, Firth of Forth, Scotland.	Harris, M.P., Wanless, S., Barton, T.R. (1996). Site use and fidelity in the common guillemot *Uria aalge*.	Ibis 133: 153-161.
Isle of May, Firth of Forth, Scotland.	Wanless, S., Harris, M.P., Greenstreet, S.P.R. (1998). Summer sandeel consumption by seabirds breeding in the Firth of Forth, south-east Scotland.	Ices Journal of Marine Science 55: 1141-1151.
Isle of May, Firth of Forth, Scotland	Rindorf, A., Wanless, S., Harris, M.P. (2000). Effects of changes in sandeel availability on the reproductive output of seabirds.	Mar. Ecol. Prog. Ser. 202 : 241-252.

Sumburgh Head, Shetland.	Monaghan, P., Walton, P., Wanless, S., Uttley, J.D., Burns, M.D. (1994). Effects of prey abundance on the foraging behaviour, diving efficiency and time allocation of breeding guillemots *Uria aalge*.	Ibis 136: 214-222.
Sumburgh Head, Shetland.	Uttley, J.D., Walton, P., Monaghan, P., Austin, G. (1994). The effects of food abundance on breeding performance and adult time budgets of guillemots *Uria aalge*.	Ibis 136: 205-213.
British and Irish colonies	Rothery, P., Wanless, S., Harris, M.P. (1988). Ananlysis of counts from monitoring guillemots in Britain and Ireland.	Journal of Animal Ecology 57: 1-19.
British east coast.	Camphuysen, C.J., Webb, A. (1999). Multi-species feeding associations in North Sea seabirds: jointly exploiting a patchy environment.	Ardea 87 (2): 177-198.
Central North Sea	Camphuysen, C.J. (1998). Diurnal activity and nocturnal group formation of wintering common murres in the Central North Sea.	Colonial Waterbirds 21 (3): 406-413.
Belgian coastal waters.	Offringa, H. & Meire, P. (1996). Verhouding zeekoet *Uria aalge*/alk *Alca torda* in Vlaamse kustwaren.	Mergus 10: 333-344.
Northeast Spain and Western Mediterranean.	Carboneras, C. (1988). The auks in the Western Mediterranean.	Ring. & Migr. 9: 18-26.
St. Lawrence Island, Bering Sea, Alaska.	Johnson, S.R., West, G.C. (1975). Growth and development of heat regulation in nestlings, and metabolism of adult common and thick-billed murres.	Ornis Scand. 6: 109-115.
Bering Sea.	Coyle, O., Hunt, G.L., Decker, M.B., Weingartner, T.J. (1992). Murres foraging, epibenthic sound scattering and tidal advection over a shoal near St. Georges Island, Bering Sea.	Mar. Ecol. Prog. Ser. 83: 1-14.
Gulf of Alaska.	Piatt, J.F. & Nettleship, D.N. (1985). Diving depths of four Alcids.	Auk 102: 293-297.
Gulf of Alaska.	Piatt, J.F. & Van Pelt, T.I. (1997). Mass-mortality of guillemots (*Uria aalge*) in the Gulf of Alaska in 1993.	Mar. Pollut. Bull. 34: 656-662.
Pribilof Islands, Alaska.	Choe,J.C., Kim, K.C. (1987). Community structure of arthropod ectoparasites on Alaskan seabirds.	Can. J. Zool. 65: 2998-3005.

Pribilof Islands, Alaska.	Decker, M.B. & Hunt, G.L. (1996). Foraging by murres (*Uria* spp.) at tidal fronts surrounding the Pribilof Islands, Alaska, USA.	Mar. Ecol. Prog. Ser. 139: 1-10.
Washington Peninsula, USA.	Parrish, J.K. (1995). Influence of group size and habitat type on reproductive success in common murres (*Uria aalge*).	Auk 112: 390-401.
Yaquina Bay, Oregon coast, USA.	Bayer, R.D. (1986). Seabirds near an Oregon estuarine salmon hatchery in 1982 and during the 1983 *El Nino*.	Fishery Bulletin 84 (2): 279-286.
Oregon coast, USA.	Bayer, R.D., Lowe, R.W., Loeffel, R.E. (1991). Persistent summer mortalities of common murres along the Oregon Central Coast.	The Condor 93: 516-525.
Farallon Island, California, USA.	Sydeman, W.J. (1993). Survivorship of common murres on Southeast Farallon Island, California.	Ornis Scandinavia 24 (2): 135-141.
Gulf of Farallones, California, USA.	Ainley, D.G., Spear, L.B., Allen, S.G., Ribic, C.A. (1996). Temporal and spatial patterns in the diet of the common murre in California waters.	The Condor 98: 691-705.
Newfoundland, Canada.	Montevecchi, W.A. & Piatt, J. (1984). Composition andd energy contents of mature inshore spawning capelin (*Mallotus villosus*): implications for seabird predators.	Comp. Biochem. Physiol. 78A: 15-20.
Newfoundland, Canada.	Schneider, D.C. & Piatt, J.F. (1986). Scale-dependent correlation of seabirds with schooling fish in a coastal ecosystem.	Mar. Ecol. Prog. Ser. 32: 237-246.
Newfoundland, Gull Island, Canada.	Cairns, D.K., Bredin, K.A., Montevecchi,W.A. (1987). Activity budgets and fraging ranges of breeding common murres.	Auk 104: 218-224.
Newfoundland, Canada.	Piatt, J.F. & Nettleship, D.N. (1987). Incidental catch of marine birds and mammals in fishing nets off Newfoundland, Canada.	Mar. Pollut. Bull. 18 (6B): 344-349.
Newfoundland, Canada.	Bartlett, S.E., Threlfall, W. (1987). A comparison of the karyotypes of six species of Charadriiformes.	Genome 29: 213-220.
Newfoundland, Gull Island, Canada.	Cairns,D.K., Montevecchi,W.A., Birt-Friesen,V.L., Macko,S.A. (1990). Energy expenditures, activity budgets, and prey harvest of breeding common murres.	Studies of Avian Biology 14: 84-92.
Newfoundland, Gull Island, Canada.	Burger, A.E. & Piatt, J.F. (1990). Flexible time budgets in breeding common murres: buffers against variable prey abundance.	Studies in Avian Biology 14: 71-83.

Newfoundland, Witless Bay, Canada.	Piatt, J.F. (1990). The aggregative response of common murres and Atlantic puffins to schools of capelin.	Studies in Avian Biology 14: 36-51.
Newfoundland, Canada.	Elliot, R.D., Collins, B.T., Hayakawa, E.G., Métras, L. (1991). The harvest of murres in Newfoundland from 1977-78 to 1987-88.	In: Gaston, A.J. & Elliot, R.D. (Eds). Studies of high latitude seabirds. 2. Conservation biology of the thick-billed murres in the Northwest Atlantic.
Newfoundland, Canada.	Friesen, V.L., Montevecchi, W.A., Davidson, W.S. (1993). Cytochrome *b* nucleotide sequence variation among the Atlantic Alcidae.	Hereditas 119: 245-252.
Newfoundland, Great Island, Canada.	Regehr, H.M. & Rodway, M.S. (1999). Seabird breeding performance during two years of delayed capelin arrival in the northwest Atlantic: a multi-species comparison.	Waterbirds 22 (1): 60-67.
Newfoundland, Canada	Rowe, S., Jones, I.L., Chardine, J.W., Elliot, R.D., Veitch, B.G. (2000). Recent changes in the winter diet of murres (*Uria spp.*) in coastal Newfoundland waters.	Canadian Journal of Zoology 78 : 495-500.
Vancouver Island, Canada.	Logerwell, E.A. & Hargreaves, B. (1996). The distribution of sea birds relative to their fish prey off Vancouver Island: opposing results at large and small scales.	Fish. Oceanogr. 5: 163-175.
Gannet Islands, Labrador, Canada.	Birkhead, T.R. & Netlleship, D.N. (1987). Ecological relationships between common murres, *Uria aalge*, and thick-billed murres, *Uria lomvia*, at the Gannet Islands, Labrador. I. Morphometrics and timing of breeding.	Can. J. Zool. 65: 1621-1629.
Gannet Islands, Labrador, Canada.	Birkhead, T.R. & Netlleship, D.N. (1987). Ecological relationships between common murres, *Uria aalge*, and thick-billed murres, *Uria lomvia*, at the Gannet Islands, Labrador. II. Breeding success and site characteristics.	Can. J. Zool. 65: 1630-1637.
Gannet Islands, Labrador, Canada.	Birkhead, T.R. & Netlleship, D.N. (1987). Ecological relationships between common murres, *Uria aalge*, and thick-billed murres, *Uria lomvia*, at the Gannet Islands, Labrador. III. Feeding ecology of the young.	Can. J. Zool. 65: 1638-1649.

Table b : list of studies referring to the guillemot's ecology through the North Atlantic.