North Sea seabirds and marine mammals: pathology and ecotoxicology

SUSTAINABLE MANAGEMENT OF THE NORTH SEA

SCIENTIFIC SUPPORT PLAN FOR A SUSTAINABLE DEVELOPMENT POLICY

BELGIAN SCIENCE POLICY

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Programme: "Sustainable management of the North Sea"

North Sea seabirds and marine mammals : pathology and ecotoxicology.

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GENERAL INTRODUCTION

The North Sea: environmental problems and pollution.

With highly industrialised 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, 1993b, OSPAR Commission, 2000). 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. The intensive use of the North Sea causes a number of problems in relation to a healthy ecosystem and sustainable use: a series of old problems continues to affect the ecosystem, sometimes showing some signs of amelioration whereas new problems have also occured (i.e. detection of new synthetic compounds) (OSPAR Commission, 2000).

Considerable efforts to understand the biogeochemistry of pollutants in the marine environment have been made over the last decades. Time trend analysis of pollutant inputs, and in particular for persistent organic pollutants (POPs) and 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 and 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. Although a general reduction in concentrations of trace organic contaminants has been observed in the North Sea, an increasing number of synthetic compounds is detected, for which the ecological effects are largely unknown (OSPAR Commission, 2000).

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 (Philippart, 1998 ; Ducrotoy, 1999; Frid and Hall, 1999; Lindeboom and de Groot, 1999; Wolff, 2000a, 2000b) .

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 took place (Bremen, 1984; London, 1987; The Hague, 1990; Copenhagen, 1995; Lisbon, 1998) to discuss this international joint effort towards the protection, preservation and sustainable management of the North Sea environment (Tromp and Wieriks, 1994). All coastal states around the North Sea have ratified the Ramsar Convention on Wetlands of International Importance. Many of these wetlands 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). In addition to the Ramsar Convention, Belgium has also ratified the Agreement on the Conservation of Small Cetaceans of Baltic and North Sea (ASCOBANS). Furthermore, in accordance with the European Commission Habitats Directive, Belgium has also proposed a large part of the western part of the coast to be included in the Natura 2000 network as a 'Special Area for Conservation'

Seabirds in the North Sea.

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 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 and Becker, 1992; North Sea Task Force, 1993a, 1993b; OSPAR Commission, 2000). 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 (waders and ducks), also find a sheltering area within the Southern North Sea and feed on mud flats 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 and Becker, 1992; Carter et al. 1993; Skov et al. 1996).

Beached Bird Surveys (BBS).

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 (Burger and Fry, 1992 ; Camphuysen et al. 1999 ; Wiese and Ryan, 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.

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' (BBS) 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 (Kuyken, 1978; Camphuysen, 1989; Camphuysen and van Franeker, 1992; Skov et al. 1996).

In Belgium, beached bird monitoring started in the late 1950s with standardized surveys from 1962 onwards (Seys, 2001). Occasionnal land and ship based seabird at sea counts, 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, 1983b). More systematic surveys of the Belgian coastal zone 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 Mathematical Models of the North Sea and the Scheldt Estuary (MUMM, Belgian Ministry of Public Health and Environment) (Offringa et al. 1996).

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, lack of food, diseases and parasites) and/or to artificial conditions such as large oil spills, 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 (Furness, 1989 ; Dorrestein and van der Hage, 1997 ; Camphuysen et al. 1999). The common guillemot is the most common species among those found stranded at the Belgian coast (Kuyken, 1978 ; Sheridan and Palmart, 1988 ; Seys and Meire, 1992 ; Jauniaux et al. 1993, 1996, 1998 ; Seys, 2001). Other typical species, but far less numerous are great crested grebes razorbills, fulmars, herring gulls, black-headed gulls, kittiwakes and oystercatchers.

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 and Underwood, 1984; Camphuysen, 1989, 1998b; Piatt et al. 1990; Bodkin and Jameson, 1991; Camphuysen and van Franeker, 1992; Harris and Wanless, 1996; Piatt and Ford, 1996; Skov et al. 1996; Oka and Okuyama, 2000). Large numbers were associated to important shipwrecks (Torrey Canyon, 1967; Amoco Cadiz, 1978; Exxon Valdez, 1989 in Alaska; Braer, 1993 in Shetlands; Sea Empress, in Wales, 1996 and the Erika, 1999 in Brittany) 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). In the Netherlands, 89 % of guillemots were oiled between 1969 and 1985 (Camphuysen, 1989), while 76 % were oiled from 1986 to 1995 (Camphuysen, 1995). Beached bird surveys along the Belgian coast revealed that, from 1962 to 1977, 97 % of guillemots were oiled (Kuyken, 1978; Verboven, 1979).

Apart from mineral oils, unusual strandings due to other non-mineral oil (nonylphenol, dodecylphenol, bis-phenol, vegetable oil, paraffin waxes) have been recorded. This was the case in The Netherlands (Zeeland) 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) (Camphuysen et al. 1998). Carcasses presenting similar characteristics were also recorded at the Belgian coast in 1998 (J. Seys, pers. com.).

When basic activities such as foraging are compromised due to low food availability, emaciated and exhausted birds rapidly wreck. Starvation, in combination with cold stress, is one of the 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, 1990b). Collapse of sprat 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 during the winter of 1986/87, where thousands of emaciated guillemots were washed ashore after a rapid decline in Barents Sea capelin (Barrett and Krasnov, 1996).

Seabird threats.

Incidental entanglement in fixed gillnets is yet another threat to diving seabirds. In the North Sea, only few studies reporting this kind of mortality exist (Murray et al. 1994; Lorentsen and 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 and 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 nets in order to reduce seabird death 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 (Dunnet et al. 1990). Similarly, all sorts of plastic debris (small plastic spherules, condom and fishing line) were described by Jauniaux et al. (1996) from the gizzard of 1 guillemot, 3 fulmars and 1 herring gull 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 (Walsh, 1990; Furness, 1993; Nisbet, 1994; Furness and Camphuysen, 1997). 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 (Scheuhammer, 1987; Furness and Greenwood, 1993). Multidsciplinary ecotoxicological studies are viewed as an interesting tool to understand the influence of pollutants and their long-term effects on the marine environment (Luoma, 1996).

These 4 above described features: abundant, pelagic, top predator, and vulnerable to oil make the common guillemot a key species to evaluate the North Sea seabirds health status. Common findings on stranded seabirds necropsies include severe emaciation, acute haemorrhagic gastro-enteritis, oil contamination, food shortage, plastic ingestion (von Petermann et al. 1989; Camphuysen and van Franeker, 1992; Camphuysen and Leopold, 1994; Jauniaux et al. 1996). However, published information remains fragmentary and oriented towards one single type of evaluation such as pathology or the toxicology. In rehabilitation centers, the same lesions are present but a higher incidence of fungal diseases is observed (Jauniaux and Coignoul, 1994). In case of oil spills, usually birds are severely oiled, but in good nutritional status, indicating that the animal died quickly.

Marine mammals in the North Sea.

The harbour porpoise is by far the most common species of marine mammals in the Northeast Atlantic and the North Sea (Hammond et al. 1995). The harbour seal is one of the most widely distributed seal in the world and the North Sea contains around 10 % of the world population (North Sea Task Force, 1993a). White-beaked dolphins are generally concentrated in a band accross the North Sea between 55° and 60° N, mostly to the west along the the eastern British coast (Hammond et al. 1995). However, extended movements may occur and groups are regularly observed in the Southern

North Sea (Haase, 1987 ; database MUMM). Other species like white-sided dolphins, hooded seals, sperm whales and fin whales can be sighted or occasionnaly found stranded but are considered as very rare in the North Sea southern bight. The North Sea is a complex ecosystem of sand banks, mudflats, sandy islands and estuaries making it unfavourable to such oceanic species (Camphuysen and Winter, 1995 ; Hammond et al. 1995).

Conservation and main threats.

Although hunting has stopped and legal protection increased (IWC, Ascobans, the North Sea Conferences, the European Habitat Directives) the populations are currently far from being unaffected by human activities, such as disturbance, change in physical habitats and/or destruction of habitats, oil spill damages, net entanglements, interactions with fisheries and environmental pollution.

Incidental entanglement and mortality in fishing gear is a global problem affecting many species of small cetacean. The effects of bycatch are probably the most important human adverse impact to small cetaceans, especially to harbour porpoises (De Jong et al. 1999, OSPAR Commission, 2000). Taking into account abundance estimates, estimated bycatch, and harbour porpoise biology, it is likely that the current bycatch alone poses a significant risk to the North Sea populations (OSPAR Commission, 2000). In addition to entanglement, an unknown number of marine mammals are also killed by discarded or lost fishing gear worldwide (Berta and Sumich, 1999).

Marine mammals strandings at the Belgian coast.

A large number of historical stranding data of cetaceans were collected by the Royal Institute for Natural Sciences (RBINS) and published (De Smet, 1974, 1981; Smet, 1981). In the late 1970s data were gathered by Van Gompel (1991, 1996) while the RBINS co-ordinated technical interventions and collected specimens. A decade later, stranding records are kept by the Management Unit of the North Sea mathematical Models (MUMM). In 1992, the MARIN group started its scientific research programme with the aim to meet specific obligations of the Belgian government in the framework of the North Sea Conferences and ASCOBANS. Scientific research of stranded marine mammals thus became systematic along the Belgian coast. This was also achieved by informing the general public and the coastal communities through information campaigns (Haelters 1997, 1999a, 1999b).

A recent recognition of the urgent need for a sustainable use of the environment has led many governments to establish long-term bio-monitoring 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 scheme.

Expertise and knowledge in different fields has been gathered in a unique multidisciplinary approach, the MARIN group (Marine Animals Research and Intervention Network), in order to study the potential causes of death and the health status of populations of seabirds and marine mammals found stranded at the Belgian coast and surrounding areas. Several teams including veterinary pathologists, marine birds ecologists and eco-toxicologists are among the different fields of scientific expertise which are strongly backed up by an essential technical and administrative support (Figure 1).
In particular, the MARIN group aimed at evaluating the cause of strandings and death, the effects of different pollutants on the animals as well as studying the links existing between the animal's general health status and its contaminants burden. A better knowledge of the ecology of the different species with surveys and experimental work organised at sea, completed the picture.

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 and Forbes, 1994) have often relied on top predators, pelagic seabirds and marine mammals as sentinel organisms (Hutton, 1981; Muirhead and Furness, 1988; Lock et al. 1992; Stewart et al. 1994, 1996, 1999; Wenzel and Gabrielsen 1995; Wenzel and Adelung, 1996). Many of those studies demonstrated high levels of contaminants (heavy metals or organochlorines) in the animals' tissues without making reference to any pathological investigations (Reijnders et al. 1999). In addition, most of these studies were carried out on robust individuals, often shot at sea or at their breeding grounds, whereas the vast majority of the stranded seabirds and marine mammals 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 taking into account pathological and ecological data.

This multidisciplinary approach clearly is quite unique at the North Sea level. Such an approach is also part of the recommendations listed by the International Whaling Commission (IWC).

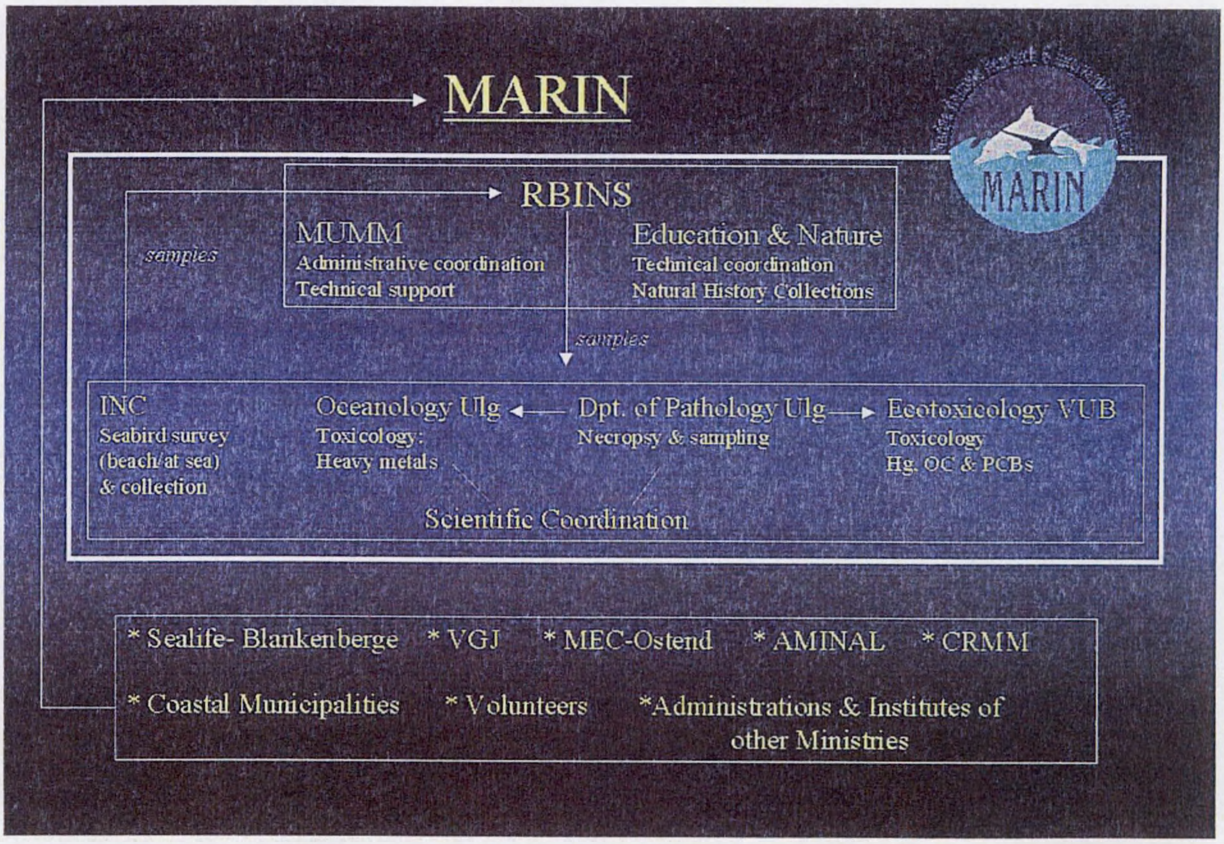


Figure 1: Organisation of the MARIN group.

SEABIRDS

Beached bird surveys.

Material and methods.

Study area.

The shoreline of the Belgian coast consists of 62.1 km of sandy beaches and another 3.3 km of industrial area within the harbour piers of Zeebrugge. Four major transects were distinguished for further analysis (from west to east): French border-Nieuwpoort FRNP (14.3 km), Nieuwpoort-Oostende NPOO (16.7 km), Oostende-Zeebrugge OOZB (20.9 km) and Zeebrugge-Dutch border ZBNL (10.2 km) (Figure 2).

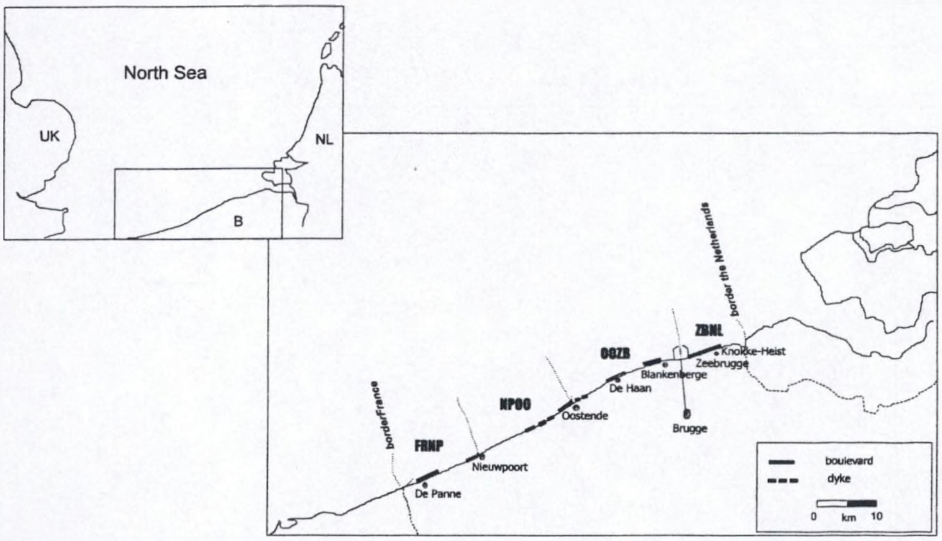


Figure 2: Study area covered with the Belgian beached-bird survey, showing the four transects used for analysis (see text for abbreviations).

For further details about the study area see Seys (2001), Seys et al. (2001), Seys et al. (submitted. a, submitted. b), and Seys et al. (accepted).

Data.

Collection of data.

Beached bird corpses were searched at the high water mark and bodies were checked for species, age, condition and (for complete corpses) the presence of oil. The output of the surveys is a list of birds containing information on the distance covered and the number of oiled and clean birds.

Beached bird surveys.

The database of Belgian beached bird surveys (BBS) consists of international, monthly, weekly and occasional surveys covering the period February 1962 – April 2001. International Beached Bird Surveys (IBBS) are carried out once a winter - generally in February - and cover ideally the entire Belgian coastline. Monthly surveys aim to take place six times a winter (October-March) along the full 65 km of Belgian beaches, with the February survey taken as the IBBS of that winter. Occasional counts are partial surveys often organised at events with mass stranding of birds (oil, wrecks, severe winter weather). Weekly surveys were started in the winter 1991-92 and carried out on the beach stretch Nieuwpoort-Oostende (16.7 km, i.e. 25 % of entire Belgian coast).

Total number of beached birds.

The total number of birds that can be collected with each of the methods mentioned above during one winter at the Belgian coast, was calculated by extrapolating and adjusting the field data for frequency and distance surveyed.

Rehabilitation centre.

Data dealing with more than 2000 moribund and injured sea- and coastal birds from the rehabilitation centre of Oostende (Marine Ecological Centre – MEC) were analysed for the winters 1988-1999 (1 October – 31 March). The majority of the birds hosted here were found on beaches between Westende and De Haan (ca. 20 km), but smaller numbers might have had their origin in the polders or on other coastal stretches. The data set includes live birds that died in care and exclude taxa considered to be essentially terrestrial (rails, passerines).

Field experiments.

Drift experiments.

At thirteen occasions during the period February 1996 - March 1999, a total of 634 bird corpses was dropped at various locations off the Belgian coast. The sample consisted for more than 90 % of auks (62 %), wildfowl (16 %) and *Laridae* (15 %). The great majority (97 %) was medium-sized (25-60 cm, Jonsson 1993). Most corpses were recovered from the freezers at the Ecomare rehabilitation centre (Texel, The Netherlands). They were all defrosted, measured and labelled with a wing-tag and a metal ring. The corpses were dropped at sea, usually in batches of ten specimens each, at various distances from the shoreline.

Persistence experiments.

During weekly winter surveys in 1997, 1998 and 1999 on the beach section Oostende-Nieuwpoort, 562 bird corpses were tagged (with an inconspicuous label around the leg) and searched after at consecutive surveys. We defined persistence time as the mean number of days a corpse could be recovered on the beach, before washing away, getting buried by sand or being taken by predators or men.

Beached bird surveys in Belgium for five successive winters 1997-2001: general results.

From winter 1996-1997 till last winter a total number of 3362 birds were counted during beached bird suveys at the Belgian coast. The effort varied between 530 km in 2000-01 and 715 km 1999-2000 of walking on the beach. Highest beached numbers were found during winter 1998-99 (>30 % of total), while less than 200 birds were found in winter 2000-01. The most common species was the guillemot, with highest numbers beached in 1998-99. Other taxa with high numbers are gulls and waders, with peaks in respectively 1999-2000 and 1996-97 (Table I).

Species/taxon	***1996-97***	***1997-98***	***1998-99***	***1999-00***	***2000-01***	***Overall***
Guillemot	0,169(74,3)	0,230(66,1)	0,924(45,6)	0,387(72,4)	0,108(58,8)	**0,378**(57,6)
Razorbill	0,039(75)	0,029(63,6)	0,079(69,8)	0,083(76,1)	0,004(50)	**0,050**(72)
Northern gannet	0,011(16,7)	0,011(40)	0,026(90,9)	0,031(37,5)	0,004(0)	**0,017**(47,5)
Fulmar	0,019(12,5)	0,067(20)	0,247(21,1)	0,063(23,1)	0,015(12,5)	**0,085**(20,7)
Scoters	0,034(76,5)	0,020(60)	0,057(73,3)	0,042(55,6)	0,011(33,3)	**0,034**(65,5)
Divers	0,005(100)	0,007(66,7)	0,015(88,9)	0,007(75)	0,006(0)	**0,008**(72,7)
Gulls	0,339(12)	0,257(25,2)	0,294(36,1)	0,373(27,3)	0,128(2,2)	**0,285**(22,6)
Waders	0,822(5)	0,025(8,3)	0,018(25)	0,014(14,3)	0,028(0)	**0,173**(5,5)
others	0,175(10,8)	0,054(29,2)	0,099(40,5)	0,106(25,6)	0,045(15)	**0,097**(22,2)
OVERALL	**1,613**(17,1)	**0,700**(42,3)	**1,758**(43,1)	**1,105**(50,8)	**0,350**(25,3)	**1,128**(36,4)
EFFORT (km)	566,7	552,5	618,2	713,8	529,2	2980,4

Table I: Densities (N/km) and overall oilrate of different sea- and coastal bird species and taxa at the Belgian coast for five successive winters (1997-2001) (between brackets : % oilrate; effort : total length of distance travelled on the beach).

Each winter can be caracterised as follows (Table I):

- winter 1996-97 : high densities of dead waders and gulls (both cold victims) and high oilrate of guillemot, razorbill, scoters and divers;
- winter 1997-98 : winter in between the others, no highlights in total numbers, oil rates and densities;
- winter 1998-99 : high densities of guillemots and fulmars, two typical wreck-species; lowest oilrate for the guillemot for the five winters because of wreck in february 1999 (more information about this winter : see also Seys et al. 1999), also high oilrate for Northern gannet;
- winter 1999-00 : high densities of guillemots (typical wreck-species), razorbill and gulls, highest overall oilrate because of several oil-incidents in the Belgian marine waters;
- winter 2000-01 : low overall oilrate (again mainly because of high proportion of gulls); low overal density (only 5 % of all birds detected on the beach during these five winters (N = 185)); also very low proportion of other species/taxa.

Beached bird surveys in Belgium during1962-1999: a presentation of the data (after Seys et al. 2001).

Introduction.

Over the period 1962-1999, a grand total of 15,368 bird corpses (105 species) and 3 sea mammals (2 species) have been collected on Belgian beaches. With almost 8,000 km of beach travelled during these surveys, an overall mean winter density of 1.9 bird corpses.km^{-1} was calculated. Although the past ten years the effort in beached bird surveying was significantly enhanced (65 % of all distance travelled), only 45.5% of all bird carcasses were collected in this period.

The most frequently encountered sea- and coastal bird species were guillemot (N=3703: 24.1 %), large *Laridae* (N=1831: 11.9 %), small *Laridae* (N=1537: 10 %), kittiwake (N=1413: 9.2 %), common scoter (N=1260: 8.2 %), razorbill (N=1019: 6.6 %), fulmar (N=735: 4.8 %), grebes (N=607: 3. 9 %), divers (N=197: 1.3 %) and gannet (N=182: 1.2 %). Other important taxa include waders (N=680: 4.4%), passerines (N=304: 2.0 %) and rails (N=395: 2.6 %).

Target species: the common guillemot.

During auk wrecks or oil-incidents, densities of beached guillemot can easily exceed 2 birds.km^{-1}, with local peak values of 4-6 specimens.km^{-1}.

Mean oil-rates strongly declined during the study-period 1962-1999. The majority was oiled in the '60s and '70s, in the '90s 'only' 57 % of the guillemots showed external signs of oil-contamination (Spearman Rank correlation $R = -0.604$; $N = 33$; $p < 0.001$). Mean densities were highest in the '80s as a result of increased numbers and food-shortage in the Southern North Sea from 1981 onwards (Heubeck et al. 1992) (Figure 3).

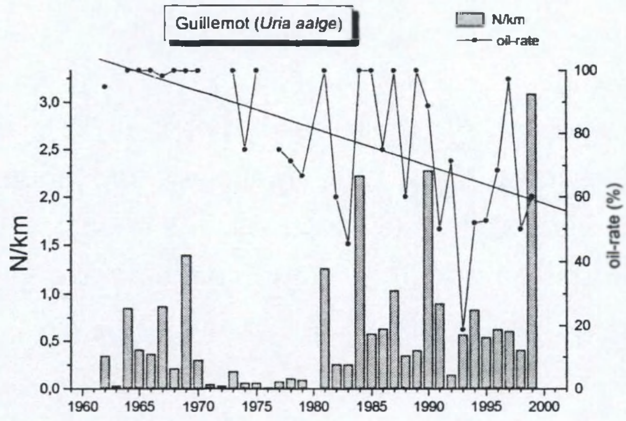


Figure 3: Density and oil-rate of beached guillemot during IBB surveys at the Belgian coast 1962-1999 (line fitted by eye).

At the MEC the guillemot was the most common species, with 1170 birds recorded over 12 winters. Oil-rates of guillemots received here during 1988-99 were markedly higher (89 %,with a range of 74-98 %) than oil-rates observed on beached corpses (57 % during the '90s) (Raevel 1992; Seys et al. 2001). Peak-periods at the MEC largely coincide with high numbers of corpses on the beach (Figure 4).

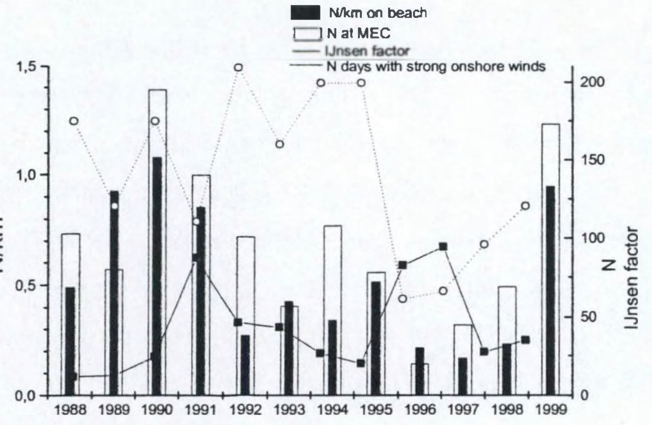


Figure 4: Density of beached guillemot and number of specimens hosted at the MEC-rehabilitation centre during 1988-99, compared to winter severity (IJnsen factor) and frequency of strong onshore winds.

An evaluation of beached bird monitoring techniques **(adapted from Seys et al. accepted).**

Introduction.

A constant and high survey effort on 65 km of sandy beach in Belgium during the winters of 1993-1999 provides data for a critical analysis of various monitoring approaches. Data of beached birds collected at the MEC and on the beach during weekly (stretch Nieuwpoort-Oostende), monthly (entire coastline) and February (IBBS) surveys are compared.

Data handling and analysis.

The sensitivity of each method in detecting events of important bird stranding on the beach (wrecks, increased stranding due to oil-slicks and winter mortality of frost-sensitive species) was analysed by ranking the top-12 densities for guillemot, northern fulmar, kittiwake or waders (as taxon) over the entire study period, grouping these high densities for each species into well-demarcated periods (events) and checking which of the four methods was able to detect the event.

Results and discussion.

Oil-rates derived from Belgian rehabilitation centres are strongly biased to oiled auks and inshore bird species. Weekly surveys on a representative and large enough section give the most reliable data on oil-rates, estimates of total number of bird victims, representation of various taxonomic groups and species-richness and are most sensitive in detecting events quickly (wrecks, oil-slicks and severe winter mortality). Monthly surveys give comparable results, although they overlook some important beaching events and demonstrate slightly higher oil-rates, probably due to the higher chance to miss short-lasting wrecks of auks. A single 'international beached bird survey' in February gives reliable data on total victim number (once the mean ratio between numbers in various months is known) and oil-rate (if a sufficiently large sample can be collected), but fails in tracking events.

Long-term changes in oil pollution off the Belgian coast: evidence from beached bird monitoring **(adapted from Seys et al. submitted a).**

Data handling and analysis.

For the study-period 1962-99, the February counts in the Belgian IBBS database covered a major part of the entire coastline. Trends in oil rates were calculated after logit-transformation of the data by means of linear regression (by least-squares estimation). The probability that a trend, if present, will be detected as statistically significant, is studied by means of a power analysis (Camphuysen, 1995). Clusters of closely related years or taxa are revealed with multidimensional scaling (Kruskal and Wish 1978), which was applied on bird densities by mapping distances in pairs of Bray-Curtis similarities between years.

Results and discussion.

Overall trend in density and oil rate of beached birds.

The total density of beached birds does not show a significant linear trend during the study-period (r^2 = 0.041, *rms* = 11.7, *b* = -0.06, *N* = 37, *p* = 0.23). However four discrete periods can be demonstrated, coinciding more or less with the four decades.

A MDS of Bray-Curtis similarities between densities with 18 taxa in 37 years confirmed this global picture (stress = 0.141). Ignoring some deviating years (like 1963, 1979, 1996), we find four clusters that agree for the greater part with our postulation (Figure 5). Taxa are clustered according to their sensitivity to severe winter weather (e.g. coot, waders) and the likelihood to occur in 'wreck'-conditions (e.g. kittiwake, guillemot, fulmar) (Figure 6). Best discriminating taxa between the assumed clusters were scoters, *Laridae*, kittiwake and guillemot.

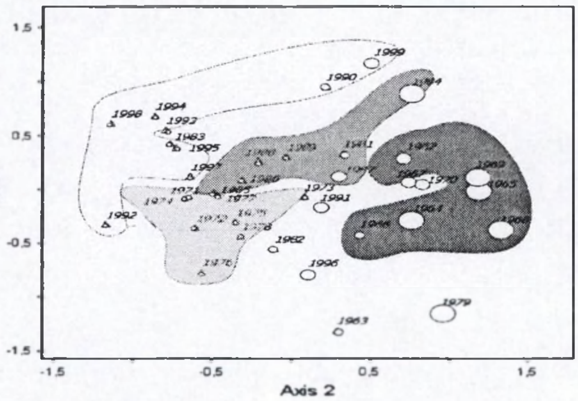


Figure 5: MDS ordination of total densities of beached birds during 1962-99, indicating clusters of winters (fitted by eye). Circle sizes reflect total densities.

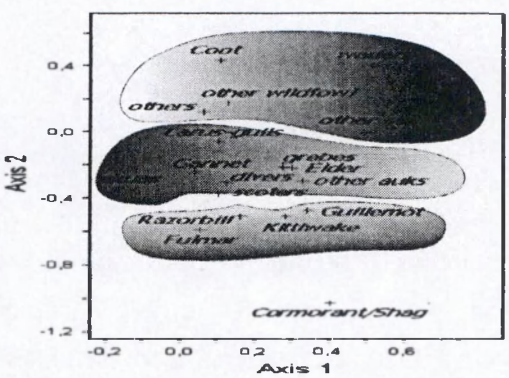


Figure 6: MDS ordination of total densities of beached birds during 1962-99, indicating clusters of taxa (fitted by eye)

Onshore versus offshore bird taxa.

Densities of *Laridae*, northern gannet, scoters and divers significantly decreased at IBB surveys during 1962-99. The guillemot is the only common species that became significantly more abundant during the study-period. Taxa or species that do not show significant trends appear to be either offshore species (fulmar, kittiwake, skuas) or birds known to be sensitive to cold winter weather (coot, grebes, waders).

Oil rates of two typically offshore species, the fulmar and kittiwake, have not changed significantly during the 37 years of study, although lowest values have been recorded for both species in the 1990s. Two groups of birds that are very common at 10-30 km from the coast, the *Laridae* and the guillemot, show significant declines in oil rate. For the other taxa no significant decrease in proportion of oiled birds could be demonstrated, often due to the insufficient number of birds yet collected.

Trends were often not significant due to the small sample size. A power analysis revealed that one needs at least 50 years of surveying to get a 90 % probability to find an existing trend for waders, grebes and scoters (Figure 7). Only for razorbill (17 years), Larus-gulls (29 years) and guillemot (31 years), the IBB surveys can already produce significant trends.

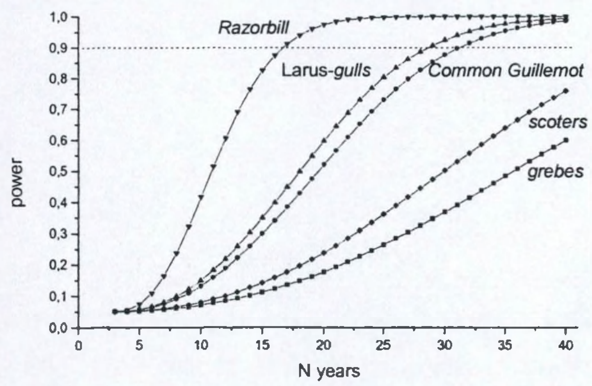


Figure 7. Power of trend test of oil-rates in seabird taxa versus number of years sampled based on IBB surveys at the Belgian coast in February during 1962-1999.

The relationship between mortality of seabirds in the Southern North Sea and densities of beached bird corpses: a review **(adapted from Seys et al. submitted b).**

Introduction.

To estimate the total mortality at sea, one needs to understand the entire pathway a seabird follows from the moment it dies, until a surveyor finds it on the beach (Figure 8). Provided we can quantify this wide spectrum of variables and relationships and we know the search effort, it will be possible to quantify (and model) the actual bird mortality at sea from the number recovered on beaches. For further details of these results we refer to Seys et al. (submitted. b).

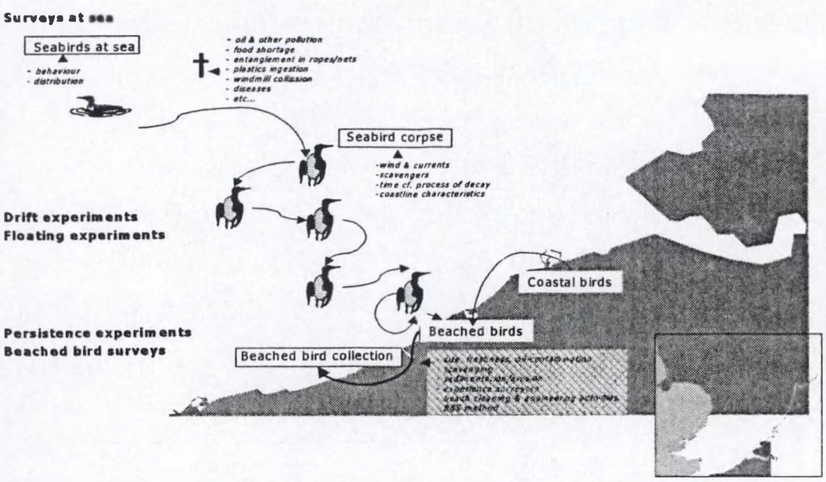


Figure 8. Conceptual pathway of a sea- and coastal bird dying in Belgian coastal waters.

Data handling and analysis.

We correlated the density of birds collected during weekly surveys with the 'wind-index' of the ten preceeding days. The abundance of seabirds at sea is assessed during ship-based surveys, carried out in the southernmost part of the North Sea since September 1992 (Seys, 2001). The focal area includes the Straits of Dover and the Belgian marine waters. During the study-period September 1992 – December 1998, more than 20,000 km had been surveyed in winter time (55 % within Belgian marine waters). We investigated whether seasonal differences in beaching numbers were in line with the temporal distribution of seabirds at sea.

Results and discussion.

Drift experiments.

Overall, 12 % of all 634 bird corpses dropped off the Belgian coast were recovered on Belgian, French or Dutch beaches. Recovery rates were highly variable. Generally the drift pattern is in accordance with the wind climate (direction and velocity) in the days after the dropping. The closer to the coast the corpses are released, the higher the probability that they will wash ashore. The more offshore a bird carcass is dropped, the more likely that it will make a long journey. The size of the bird - expressed in terms of wing-length- doesn't seem to have an impact on the travelled distance. However, longer-winged birds have a much higher probability to be recovered on the beach.

Comparison of seabird distribution at sea and density of beached birds.

In most species the relative density of beached birds on the Belgian shoreline follows the temporal pattern of occurrence at sea, as observed during ship-based surveys along fixed ferry routes (Figure 9). Often a time lag of at least one month between both peak densities can be observed. Species and taxa are more likely to have a random distribution in beaching when they occur further offshore.

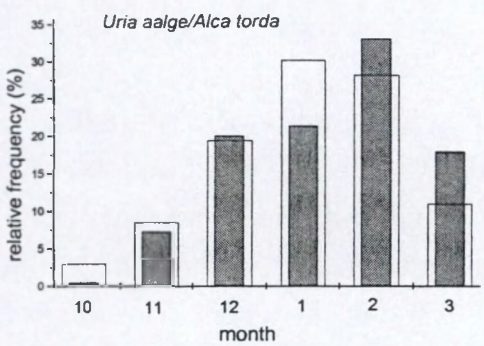


Figure 9: Relative monthly abundance of auks during ship-based surveys (hollow bars) in the southernmost part of the North Sea, compared to relative density at beached bird surveys (grey bars) in Belgium.

Persistence experiments.

On average a bird corpse remains on the beach for 5.8 – 13.3 days. Although weekly controls do not allow defining persistence time more precisely, the minimal value is probably the more realistic one. Persistence time is primarily determined by the size of the bird. A small-sized corpse has a minimal persistence time of only 0.7 days, medium-sized birds (including auks, seaducks, fulmar, most gulls) 5.5 days, large birds (gannet, great cormorant, divers, great black-backed gull, grey heron) persist on average 13 days. Oiled seabird corpses appear to have a higher but non-significant chance to disappear earlier than un-oiled birds ($F_{3,440}=0.673$; $P<0.05$). In fact only very heavily (>50 %) oiled birds will remain on the beach markedly longer (12.3 days), than all other bird corpses (6.4 days).

Conclusions.

Oil-contamination is still a major cause of mortality in many coastal and seabird species around Europe. In a comparison with other North Sea areas, oil-rates of most Belgian beached bird species are significantly higher than in Northern areas such as the Shetlands and Norway, and more or less in line with oil-rates at other European continental coasts (Camphuysen and van Franeker 1992, Seys et al. 2001). Wrecks of starved unoiled guillemots (and other species) became an almost annual event at the North Sea coasts from the first half of the '80s onwards, also in Belgium (Camphuysen 1990a, 1990b, 1990c, 1998b; Heubeck et al. 1992; Underwood and Stowe 1984; Van Gompel 1981, 1984; Verboven 1985).

Oil rates of beached bird corpses are an appropriate condition indicator of oil pollution at sea. Oilrate of most bird species/taxa in Belgium indicate a decline in oil pollution for the period 1962-99, though only *Laridae*, guillemot and razorbill show significant reductions. For the other taxa no significant decrease in proportion of oiled birds could be demonstrated, often due to the relatively small study-area and hence insufficient number of birds collected. Assuming that a sample of at least ten complete corpses is required to calculate reliable oil rates, only the guillemot (as species) and auks (as taxon) can provide the necessary data in Belgium these days (Seys 2001).

Long-term oil pollution monitoring in Belgium should be continued with a major focus on a set of abundant bird taxa, sensitive to oil-pollution and occurring in various marine habitats and the collection of additional data during the rest of the winter. Most appropriate for this set of limited bird taxa to focus on are grebes (inshore), *Laridae*, guillemot and razorbill (midshore) and kittiwake and fulmar (offshore).

Oil-pollution monitoring at sea through beach bird surveying would undoubtedly benefit from a further standardization of methods, enhancing the efficiency of data collection. Most birds collected by the Belgian rehabilitation centres were injured and still alive. Oil rates derived from these centres appeared to strongly biased to oiled auks and inshore bird species, and were therefore of little use in assessing the extent of oil pollution at sea.

The major asset of rehabilitation centres in terms of data collection seemed to be their continuous warning function for events of mass mortality. Since monthly surveys in Belgium were carried out by a network of volunteers and were spread over a larger beach section, they should be considered as the overall best performing approach. The minimal distance for a monthly survey amounts to 25-30 km (40-50 % of Belgian coastline) up to 40 km (65 %) in order to attain sound figures for oil-rate and species-richness respectively. In the near future it might be more and more difficult to collect large enough samples on the beach due to further intensification of beach cleaning in winter, even for the most abundant species.

Birds dying at sea may eventually wash ashore. As such, beached bird surveys can be an important source of information concerning mortality of seabirds in the marine environment. However, there has been a lot of debate on the question how numbers of casualties on beaches relate to the actual mortality at sea and wich factors affect this relationship. The temporal patterns of beached birds usually follow those of seabirds at sea with a time lag of at least one month. Considering the short Belgian shoreline and the prevailing frequency distribution of winds, probably only 10 % of all birds washing ashore died in Belgian marine waters. With a dominant SSW circulation and a net residual current in northeastern direction, many birds must end up on Dutch, German or Scandinavian beaches. Accordingly, there is a higher probability that Belgian beaches receive birds that died in Northern France or South-England than from other North Sea border states. Based on the number of birds found on the beach and brought in at the MEC, and taking into account that 50-80 % of the corpses have disappeared already within the first 9 days (the mean interval between succeeding weekly surveys), we estimate that the total number of bird corpses beaching on the Belgian coast each winter might be as high as 5,000-10,000 birds.

Necropsies and pathological findings.

Pathological investigations of dead seabirds provide information on the lesions and causes of death. Pathological findings must be linked to toxicological and ecological studies in order to build hypothesis on the causes of seabirds mortality in the Southern North Sea. To allow this multidisciplinary approach, the MARIN group has focused on evaluating the causes of death of stranded wintering seabirds, and particularly, of guillemots stranding along the Belgian coast.

Material and methods.

Birds collection.

Between 1997 and 2001, the Belgian beaches were systematically surveyed from November to March and biometrics measurements were taken on stranded birds. During the same period, dead birds from rehabilitation centers were also collected. Putrefied carcasses were discarded, the remaining animals were individually labeled and kept frozen for necropsy.

In addition, guillemots from Brittany were collected after the Erika oil's spill in December 1999. Finally, guillemots stranded on Scottish coastline during the 1998-1999 winter were included in your investigation.

Necropsies.

The seabirds (226 from Belgian beaches, 223 from rehabilitation centers, 96 after the Erika's spill and 23 from Scottish coastline) were necropsied using a consistent protocol (Dorrestein and van der Hage, 1993; Jauniaux et al. 1996). They were weighed, oil contamination on plumage and/or in intestinal tract was recorded and lesions were noted. A "cachexia index" was used to evaluate emaciation (1: absence of subcutaneous and abdominal fat, light atrophy of pectoral muscles; 2: absence of subcutaneous and abdominal fat, moderate atrophy of pectoral muscles; 3: absence of subcutaneous and abdominal fat, severe atrophy of pectoral muscles). Non cachectic birds, considered as "normal birds" were rated as cachexia 0. Birds were sexed by examination of gonads and age was determined by the presence (juvenile) or the absence (adult) of the bursa Fabricius. Tissues were collected and processed for histopathological, bacteriological and parasitological examinations (Jauniaux and Coignoul, 1994; Jauniaux et al. 1996; Brosens et al. 1996).

The gastro-intestinal content was tested for the presence of oil and blood. In case of internal oil contamination, characterized by presence of intraluminal dark red content, the stomach or intestine was flushed with xylene. The extraction was compared with xylene extraction of oil from feathers. In addition, the same content was tested for the presence of blood using labstick. Pectoral muscles, liver and kidneys were sampled for toxicology.

Statistical analysis.

Chi-square statistical tests using 2x2 contingency tables were performed to investigate associations between biological data (age, sex) and pathological findings on guillemots (Jauniaux et al. 1996, 1997, 1998). Associations were also analysed using ANOVA tests and differences were considered as significant for $P<0.05$. For some tests, cachectic categories 1, 2 and 3 were pooled.

Results.

Birds collection.

Along the Belgian coast, a peak density of strandings was observed in February. Biometrics measurements of guillemots stranded on the Belgian coastline indicated that most belonged to the nominate *U. aalge albionis*, breeding in Ireland, Helgoland and Southern Britain and *U. aalge aalge*, breeding in Northern Scotland (Offringa et al. 1995).

Necropsies.

Main observations (all species).

Most frequent observation, in the various groups of birds considered (Table II) were acute cachexia, haemorrhagic gastro-enteropathy (AHGE) and oil contamination. In cases of AHGE, the intestinal serosal surface was congested, the intestinal wall was thickened and hyperaemic, and haemorrhagic content was present in the lumen.

Other lesions were urate nephropathies, tissular congestion and fungal pneumonitis and aerosacculitis. Acute gastric ulcers were frequently associated with nematodes (in proventriculus and under the koilin layer of gizzard). The most frequent worm species were *Contracaecum spiculigerum, Cosmocephalus obvelatus* and *Paracuaria tridentata.*

Observations in Belgian guillemots (beached).

There were 78 males, and 72 females; 110 immatures and 40 adults. Sex and age were not determined in 5 birds, due to severe decay. The body weight and according nutritional status are reported in Table III. The main observations in decreasing order were cachexia > AHGE > oil contamination. High incidence of gastro-intestinal nematodes infestation was observed.

Observations	**Beach (All species) n=226**	**Rehabilitation centers n=223**	**Guillemots (Belgian beach) n=155**	**Guillemots (Brittany) n=71**	**Guillemots (Scotland) n=23**
Oil contamination	29,6 %	55,2 %	30,1 %	98,6 %	10 %
Cachexia	85 %	93,3 %	93,5 %	43,6 %	90 %
AHGE	66 %	68,6 %	74,2 %	45 %	70 %
Gastric ulcers	12 %	11,7 %	17,4 %	4,2 %	
Intestinal content	Normal 18% Blood 59,9% Empty 22,1%	Normal 13,8 % Blood 65% Empty 21,2%	23,9% 12,9% 57,7% 64,5% 16,9% 22,6%	Normal 23,9% Blood 57,7% Empty 16,9% Oil 1,4%	Normal 0% Blood 45% Empty 55%
Tissular congestion	23 %	38,1 %	22,6 %	82 %	35 %
Urate nephropathie	17,3 %	24,6 %	18 %	35,2 %	10 %
Fungal pneumonitis or airsacculitis	5 %	7 %	7,1 %	-	-
Gastro-intestinal nematodes	51,2 %	45,7 %	65,8 %	18,3 %	40 %

Table II: Comparison of the main observations collected on seabirds from the Belgian coastline from 1997 to 2001, from Brittany, after the Erika's oil spill in 1999 and from Scotland.

Statistical tests were performed on 149 guillemots for which all data were available. Cachexia was more frequently observed in immature birds (p = 0,0044); adults were more frequently oiled (p = 0,0017). No significant association appeared between age and other observations as well as between sex and the main observations. In addition, cachexia was associated with oil contamination, non oiled birds being more frequently cachectic (p = 0,0017). AHGE was not associated with cachexia or with oil contamination. ANOVA tests revealed that body mass was different when considering the 3 levels of cachexia but not by sex, age, AHGE or oil contamination.

	Stranded guillemots (Belgian beach)	**Severely oiled guillemots (Brittany)**	**Stranded guillemots (Scotland)**
Normal birds	863,3 g ± 125,6 (n=9)	857,2 g ± 146,3 (n = 28)	785 ± 5 (n=2)
Birds cachexia 1	723,58 g ± 87,7 (n = 31)	786,2 g ± 92,1 (n = 14)	
Birds cachexia 2	669,1 g ± 64,7 (n = 73)	651,4 g ± 85,8 (n = 10)	
Birds cachexia 3	576, 2 g ± 51,8 (n = 36)	580 g ± 68,8 (n = 16)	
Birds (all cachexia index)	657, 3 g ± 85 (n = 140)	670,2 g ± 120 (n = 40)	653 g ± 73,5 (n=18)
Maximal weight	1020 g	1318 g	
Minimal weight	480 g	489 g	

Table III: Comparison of body weight following nutritional status of stranded guillemots from the Belgium coastline (1997 to 2001) and from Brittany, after the Erika's oil spill (1999).

<u>Guillemots collected after the Erika's oil spill.</u>

There were 23 males, and 45 females; 53 immatures and 16 adults. Sex and age were not determined, respectively in 3 and 2 birds, due to severe decay. The main observations in decreasing order were oil contamination > AHGE> cachexia. Except for one bird, all were severely contaminated by oil and one was internally contaminated with oil, confirmed by xylene extraction procedure. The heavy weight of some Erika's guillemots could be due to severe oil contamination of the feathers.

Statistical tests were performed on 68 guillemots for which all data were available. The 98,6 % proportion of birds contaminated by oil impeded correlation analyses with other post mortem observations. Only one relation was significant, cachexia being frequently observed in birds with acute haemorrhagic gastro-enteropathy ($p = 0,03$). Other relations between age, sex and lesions, and between lesions were not significant. Regarding the nutritional status, no difference appears between normal and cachectic birds neither as between cachectic 2 and cachectic 3 birds.

Scottish guillemots.

There were 9 males, and 9 females; 9 immatures and 9 adults. Sex and age were not determined for 5 birds, due to severe decay. Low number of animals impaired statistical analyses. Nevertheless, as for Belgian guillemots, the main observations in decreasing order were cachexia > AHGE > oil contamination, with similar frequency. High incidence of gastro-intestinal nematodes infestation was also observed.

Comparison between Belgian, Erika's and Scottish guillemots.

Analyses revealed that guillemots from Belgian beaches presented more frequently AHGE ($p < 0,0001$) and cachexia ($p < 0,0001$) than animals from Brittany. The mean body weight (all categories of nutritional status) was 747,2 g $\pm$ 160 for Erika's and 669,7 g $\pm$ 100 for Belgian guillemots. This highly significant difference ($p < 0,0001$) was only due to a higher proportion of cachectic birds among those collected in Belgium. Observations did not differ between Belgian and Scottish stranded guillemots with a similar proportion of birds with cachexia AHGE .

Discussion.

The most frequent and most significant lesions observed were by far cachexia, a debilitating, long lasting condition, and AHGE, an acute lesion.

Cachexia and AHGE.

Body weight (Table IV) was too low in all birds, with a deficit of 35 % for cachectic birds and 25 % for so-called normal birds, when compared with a population of guillemot shot at sea in Scotland in November (Furness et al. 1994).

	Scotland		Belgium (1997-2001) Stranded(2)	France (Brittany) Oiled (2)	Belgium (1993-1994) Stranded(3)
	Shotted (1)	Stranded (2)			
Normal birds	1031 g	785 g	863,3 g	857,2 g	736 g
Cachectic birds		653 g	657,3 g	670,2 g	661 g

Table IV: Comparison of body weight following nutritional status of guillemots stranded and oiled in Scotland, Belgium and France. (1) Furness et al. 1994 ; (2) present analyses ; (3) Jauniaux et al. 1998.

It is also noteworthy that 91 % of all birds had empty digestive tracts, indicating unavailability of food or incapacity to feed. Wintering guillemots stranded along the Belgian coast were all debilitated, with empty guts and weight deficits of variable severity. Similar observations can apply to guillemots stranded along the Dutch coast when the weight range (Camphuysen, 1989) was compared to animals from Scotland. The origin of emaciation was not determined but it could have been an association of stressful situations such as food deprivation, mild chronic oil exposure, low temperature or long term effects of contaminants leading to accelerated starvation as described by Bray (1979). Experimental food-deprived gulls exposed to a single oral dose of oil lost 1/4 of their weight in 4 days (Peakall et al. 1985) while after oiling, guillemots lost 1/3 of their body weight during rehabilitation in an asylum (17 to 42 days) (Khan and Ryan, 1991). For the latter, weight loss was associated with malabsorption, impaired liver function and increased metabolic rates.

AHGE and intestinal haemorrhages have often been reported to be linked with oil exposure in the field (Pionneau, 1987; Fry and Lowenstine, 1985) but have not been experimentally reproduced (Holmes et al. 1978, 1979; Szaro et al. 1978; Leighton, 1985; 1986). Lesions could have been more the result of stress more than a direct toxic effect of oil ingestion (Leighton, 1993; Briggs et al. 1996). Consumption of petroleum-contaminated food appears to be a non-specific stressor, increasing the effects of other stresses such as low temperature (Holmes et al. 1978, 1979).

Previously, on Belgian stranded guillemots, Jauniaux et al. (1998) have reported absence of correlation between cachexia and AHGE on the one hand and visible oil on the plumage or in the gut on the other hand. These data are in contradiction with previous field observations (Jauniaux et al. 1994, 1996) and experimental work (Peakall et al. 1985). In the present study, we report on the one hand for Belgian guillemots a negative correlation between cachexia and oil contamination, non oiled birds being more frequently cachectic, and on the other hand a lower proportion of cachectic birds among Erika's guillemots. Guillemots from the Belgian coastline were less contaminated by oil on a small body surface while almost all the animals from Brittany were contaminated by oil, on the entire body surface. Similar observations have been reported, birds with extensive oil exposure being in good nutritional state, those with limited exposure being emaciated (Camphuysen, 1989; Camphuysen and van Franeker, 1992; Camphuysen and Leopold, 1994). The reason for the apparent lack of correlation between recent oil exposure and lesions can be manifold. Oil being a well known, pervasive pollutant of the Southern North Sea, mild, chronic exposure, not necessarily visible on feathers or in the gut, could lead to a chronic debilitating effects, such as cachexia, that cannot be readily linked to a precise cause. In addition, in our sample, we suspect that some guillemots stayed previously in rehabilitation centers (Jauniaux et al. 1998). Collected oiled birds are cleaned in rehabilitation centres and then released. We suppose that among those, some died but without any sign of oil contamination. To avoid such problems, we recommend that all released birds should be ringed.

Mechanisms of death.

Stressful associations such as food deprivation, mild oil exposure or long term effects of contaminants may have led to cachexia and progressive debilitation. Pollutant levels were below concentrations considered to be acutely toxic, but may have represented additional stress in the debilitation process. This mechanism of progressive debilitation should not be limited to the Southern North Sea as similar observations were done for Scottish guillemots.

In the case of severe oil contamination, guillemots will die quickly due to severe hypothermia or drowning (Albers, 1991; Leighton, 1993). For contaminated birds maintained at 4°C, the consequence is a 4 fold increase of their metabolic rate paralell to a rapid decrease of body temperature (Ekker and Jenssen, 1989a, 1989b, 1989c).

Organochlorines and PCBs (adapted from Joiris et al. 1997 and Holsbeek et al. in prep.).

Introduction.

The general PCBs contamination level of the North Sea is controlled by the circulation and flux of PCBs between the different compartments. Sediments and suspended particulate matter dispaly the highest levels of PCBs, but instead of a final sink, these compartments act as temporary reservoir until they are remobilized (Thomé et al. 1992).

PCBs which are known as persistent compound will be concentrated ten times from one trophic level to the next one (Shaw and Connell, 1986). In both terrestrial and aquatic organisms, the food is a major source of PCBs which are absorbed through the stomach walls and across the gut membranes; direct uptake through the epidermis and gills is a secondary route of uptake in the lower levels of the aquatic food chain.

Effects on wild populations.

The mobilisation of OC in birds exposed to food stress has been considered to be a factor in deaths of wild birds. In autumn 1969, more than 17,000 seabirds were washed ashore on the coastline bordering the Irish Sea. This happened at the time of the moult and most of these birds were guillemots. The number of birds recovered for that study is considered as an underestimated of the actual number of dead birds; the birds examined had no fat reserves. This wreck has been related to high PCB levels (Walker, 1990).

A marked increase in the percentage of PCBs in the liver of guillemots from that period was compared with shot individuals, i.e. from 0.9 to 22.5 %. For the birds found dead, the mean level of PCB residues was 56 ppm in liver (1 to 80) and 3.2 ppm in the rest of the body; for the shot birds the levels were 0.4 and 3.4 ppm respectively. This is because much of the PCB residues in shot birds would have been in fatty tissue whereas almost all the residues in birds found dead would have been remobilised when fat was utilised and PCBs located in other tissues, in this case mainly liver.

The fact that in both dead and shot birds the PCBs body concentration was alike illustrates the fact that the total PCBs burden in the whole body only gives an indication of potential toxicity, whereas target tissue concentrations give the best indicator of a possible toxic effect; nevertheless, there was some evidence of pathological changes in the liver and kidney similar to those found in birds dosed experimentally with PCBs, there is little evidence that toxicity of PCBs alone has been the direct cause of mortality in field conditions; the high levels reported for the dead guillemots from 1969 are not outside the levels reported in apparently healthy herring gulls on the Great Lakes (50 to 200 ppm).

Material and methods.

Organochlorines (PCBs and Organochlorine Compounds - OC- pesticides) were determined by liquid gas chromatography with capillary column and electron capture detector (Delbeke et al. 1990). PCB concentrations are expressed as individual congeners, $\sum$ congeners, as $\sum$ICES 7, corresponding to the ICES priority list (IUPAC n° 28, 52, 101, 118, 138, 153 and 180). OC and metabolites included are : alfa-, beta-, gamma-HCH, HCB, heptachlor, aldrin, dieldrin, endrin, o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT, p,p'-DDT. In most of the samples, other compounds (heptachlor-epoxide, cis-chlordane, trans-nonachlor, metoxychlor, mirex) were identified but not further quantified. HCB, which is usually expected to appear in the first elution only, partially eluted during the second run, as did occasionally p,p'-DDE at a level < 5 %. Possible influence of partial overlaps in case of p,p'-DDD and o,p'-DDT and in some cases p,p'-DDE and dieldrin were double-checked using a slightly modified temperature programme. Detection limit for a 1 g sample: 1 to 3 ng/ g dw for individual congeners (15 ng/ g dw $\sum$ congeners); 0.5 ng/ g dw for pesticides (heptachlor epoxide, aldrin, op'- and pp'- DDE, op'- and pp'-DDT, op'- and pp'-DDD). Non polar lipids were extracted with a hexane - acetone mixture (135/15) for 10 hr at 75° C in a Soxhlet apparatus; the extracted lipids were weighed after evaporation.

Results.

In total 212 pectoral muscles, 202 livers and 125 kidneys were analysed for organochlorine content. Expressed as ICES 7, PCB levels averaged 2.0 ± 1.6 μg/g dw for muscle, 5.6 ± 5.1 for liver and 2.1 ± 2.7 for kidney. OC were mainly present as ppDDE, the main metabolite and extremely stable of the major DDT component: 0.36 ± 0.31 μg/g dw muscle, 0.85±0.79 liver and 0.44±0.42. Data vary largely, basically due to seasonal differences within the same population (see: seasonal variation PCBs and Hg). For this reason, it does not make much sense trying to describe the whole of the population by median or mean values.

Organochlorine levels as a function of pathological findings.

In order to investigate possible causes of variation in OC levels within the population, comparisons were made in function of age, sex (adults), cachexia, acute haemorrhagic gastro-enteropathy (AHGE) and oiling. Statistical tests provided non-significant or limited differences involving only some tissues or only one of both ppDDE or ICES 7; in parallel with the results for heavy metals, ppDDE and $\sum$ congener levels in liver (and ppDDE in kidney) were higher in cachectic animals on both dry and lipid weight basis (Table V).

	Lipid content			ppDDE dw			□ ICES7 dw			ppDDE lw			□ ICES7		
	M	L	K	M	L	K	M	L	K	M	L	K	M	L	K
				**											
Juvenile	0.033	0.106	0.120	0.3	0.5	0.4	1.3	3.3	2.4	7.5	4.5	3.5	41	30	18
Adult	0.036	0.104	0.126	0.4	0.8	0.5	2.1	5.5	2.8	11.4	7.9	3.9	49	50	24
					**	**					**	**		**	
Male(adult)	0.039	0.116	0.127	0.4	0.5	0.3	1.6	4.1	2.8	8.9	4.3	3.8	43	40	21
Female (ad)	0.032	0.098	0.112	0.5	1.4	0.6	2.5	9.8	3.4	14.3	14.0	5.7	79	93	32
	**				**	**		**			**	**		**	
Non-cach.	0.045	0.097	0.107	0.3	0.3	0.2	2.0	1.7	2.0	6.8	3.3	1.9	40	21	22
Cachectic	0.033	0.110	0.121	0.3	0.7	0.5	1.6	4.2	2.8	8.9	6.0	3.9	48	37	22
No AHGE	0.038	0.100	0.115	0.3	0.7	0.3	1.9	3.5	2.9	8.8	7.1	3.0	50	30	23
AHGE	0.033	0.110	0.120	0.3	0.5	0.4	1.6	3.5	2.6	8.2	5.0	3.8	45	33	21
	**	**													
No oiling	0.032	0.110	0.119	0.3	0.6	0.4	1.3	3.3	2.5	8.3	4.9	3.6	45	29	19
External	0.039	0.107	0.122	0.4	0.5	0.4	2.0	3.5	2.8	8.5	4.3	3.3	46	31	23
Internal	0.037	0.092	0.112	0.4	0.8	0.5	2.0	5.0	2.8	8.6	11.3	4.0	46	52	27

Table V: Comparison of median body weight (g), lipid content (g/g dw), ppDDE and $\sum$ congeners (μg/g dw and lw) in muscle (M), liver (L) and kidney (K) in function of age, sex (adult only), AHGE and oiling; n: number of samples. **: p ≤ 0.01.

Seasonal changes in PCB concentrations.

A threefold increase in PCBs and DDE levels from November-December to March, comparable to what was earlier described and observed for mercury can be understood as a result of a higher uptake in the Southern North Sea. OC levels increased with the time spent in the Southern North Sea region as described earlier. The newest results (Figure 10) are very much in the same line as published earlier as Joiris et al. (1997).

The sharp decrease in total PCB levels from March (this study) to July (Bourne and Bogan, 1972) strongly suggests a change in contamination due to exposure via food, as discussed for mercury; this hypothesis is based on the fact that nor the body weight variations nor the tissues lipid content variations can explain such an increase in time, so food intake and excretion remain the main mechanisms determining the overall organochlorine content; egg laying would not be an important elimination route for guillemots because the eggs are laid when the most significant drop in organochlorines would have taken place.

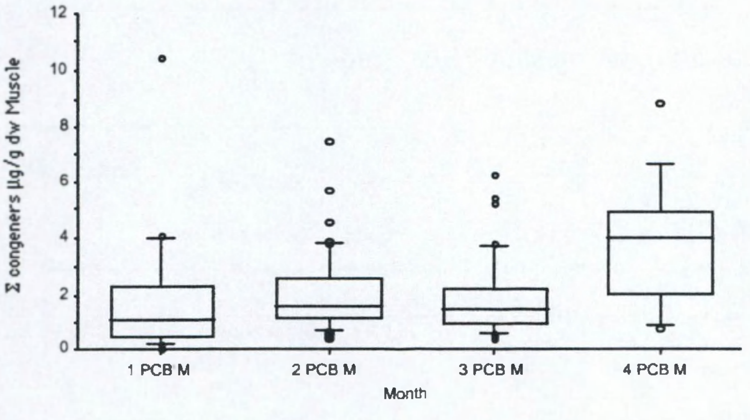


Figure 10 : PCBs as ICES 7 in muscle of common guillemot : median, 25 and 75 %, 10 and 90 % and outlayers. 1 : November-December, 2 : January, 3. February, 4. March.

Latitudinal differences in organochlorine levels.

PCB levels in the Southern North Sea are a magnitude higher when compared to data for Northern auk populations. In order to enable a reasonable comparison between our data, expressed as ICES 7 and historical data, expressed as Total PCBs (as 1260), one may consider that ICES 7 makes up for ± 30 % of a normal Aroclor 1260 mixture. Even when knowing that the patterns that are found do not entirely fit with Aroclor 1260 (see: fingerprints), a first line comparison of data shows by far higher concentrations in our Southern North Sea sampling.

Guillemots from two different locations at the Northern North Sea have been sampled by Bourne and Bogan (1972); the samples were collected in July at two different colonies, i.e. Moray Firth, Northwest Scotland and at the Faroer Islands; the total PCB levels are reported in Table VI, showing lower PCB levels in the Faroer colony compared to the Scottish colony.

If the organochlorine levels in Brünnich guillemots from Arctic waters and the black guillemots from Svalbard area are reported together with the levels from Scottish guillemots, there is still a clear latitudinal decreasing PCBs gradient.

Area	Location	Season	*n*	Muscle µg/g dw	s.d.	Liver µg/g dw	s.d.	Reference
Moray Firth, E. Scotland.	57°33'N,1°43'E 58°N, 2°22'W	July 1971	4	1.40	2.14	2.38	3.49	Bourne and Bogan,
Faroes Is. N. Scotland	61°N, 6°W 62°N, 7°30'W.	July 1971	4	0.52	0.16	0.74	0.22	Bourne and Bogan,
Spitzbergen Arctic circle	77°N, 15°E. (approx.)	not pecified 1980	4	0.24				Andersson et al. 1988.
Svalbard area	78°N, 15°E.	July	2			0.46	0.37	Daelemans et al. 1992.

Table VI: Total PCBs concentration in μg/g dw in tissues of guillemots at different areas. Mean values and standard deviation. Spitzbergen: Brünnich guillemot; Svalbard: black guillemot; other areas: common guillemot.

PCBs fingerprints.

A comparison of the percentual fingerprint (percentual contribution within ICES 7) shows that a fairly high resemblance exists between the samples and an Aroclor 1260

PCB pattern (Figure 11: Aroclor; Figure 12: average muscle, liver and kidney in common guillemot). Daelemans et al. (1992) reached similar conclusions after performing a principal component analysis between the pattern for total PCBs in black guillemot and Aroclors 1248, 1254, 1260 and 1262.

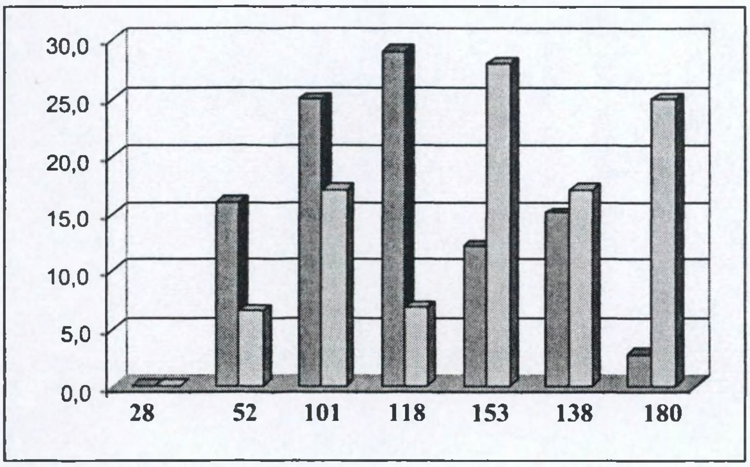


Figure 11: Aroclor relative PCBs fingerprint (ICES 7), expressed as percentage: a:1254, b: 1260.

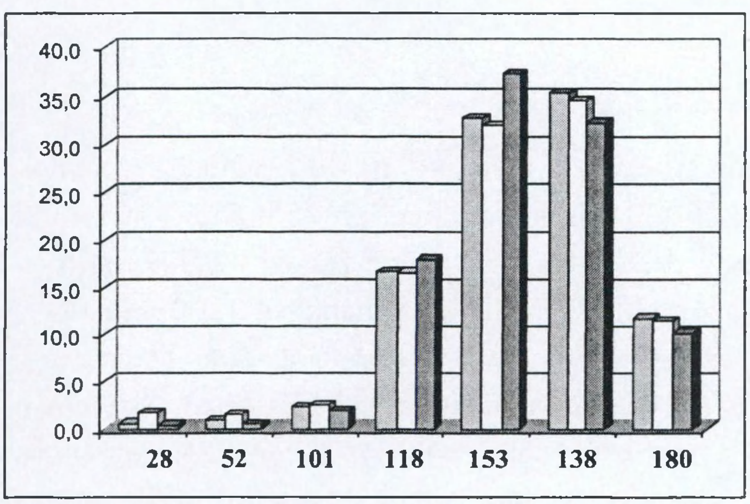


Figure 12: PCBs fingerprint (ICES 7), expressed as percentage, in common guillemots from the Southern North Sea; a. muscle, b. liver, c. kidney, average values.

Despite a large variation of concentrations within and among tissues, the overall fingerprint pattern remains highly equal throughout the entire sampling of marine birds.

Figure 13 indicates that the fingerprint pattern for guillemot differs slightly from the pattern found in e.g. sperm whale. Differences can be attributed to differences in pattern in the food, but might as well result from differences in PCBs metabolisation capacities.

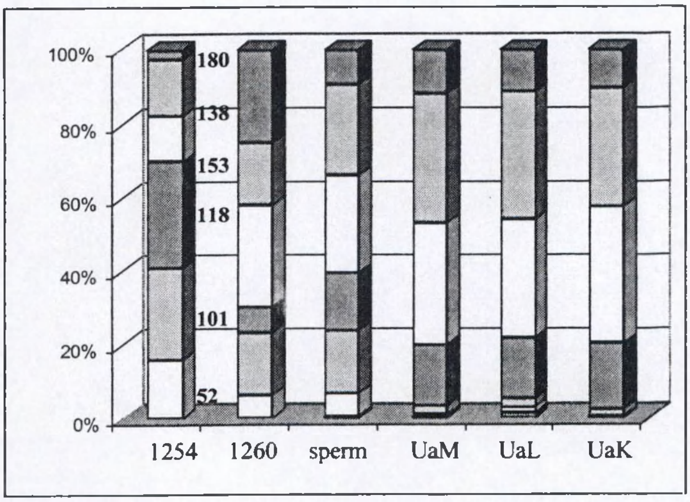


Figure 13: comparison of the relative PCBs fingerprint (ICES7), expressed as percentage: Aroclor 1254 and 1260, sperm whale blubber and common guillemot pectoral muscle (UaM), liver (UaL) and kidney (UaK).

Toxicity risk.

Toxicity data vary along species and groups and therefore, hard to evaluate. Common egrets found dying or dead, had mean PCB muscle and liver concentrations of 16 and 37 μg/g fw (approx. 67 and 132 μg/g dw) and there was no reason to attribute their death to PCBs intoxication (Faber et al. 1972). On the other hand, in 1970 and 1971, cormorants were found dead in several areas of the Netherlands and some were analysed. They contained high levels of PCBs comparable to those reported by Koeman et al. (1973) in cormorants poisoned experimentally, with PCBs liver concentrations between 210-285 μg/g fw (approx. 750-1020 μg/g dw); it is problabe that all or most of the birds from 1970-71 died from PCBs poisoning.

Guillemots from the Irish seabird masssive wreck in 1969 contained liver concentrations from 1 to 880 μg/g fw (approx. 3.5-3150 μg/g dw), it was the time of moult, there were heavy seas and the birds did not have fat reserves. There was some evidence of pathological changes in the liver and kidney similar to changes found in birds intoxicated experimentally with PCBs; it has been proposed that for the birds with liver PCBs concentration higher than 200 μg/g fw (approx. 715 μg/g dw), PCBs were actually contributing to the birds mortality (Walker, 1990). The guillemots from this study had levels of 4, 8 and 2 μg/g dw in muscle, liver and kidney. A rough recalculation would yield 'total PCB' levels of 11.8, 23.5 and 5.8 μg/g dw respectively; we cannot consider the organochlorine levels as a primary cause of mortality for the majority of the birds sampled; nevertheless, maximal 'total PCB' concentrations in liver calculated of the order of 70 μg/g dw could be responsible of ill effects for at least part of the population.

Heavy metals **(adapted from Debacker et al. 2000 and Debacker et al. 2002 in prep.).**

Introduction.

The aim of the present study is to determine the heavy metal levels in the tissues of a pelagic top predator, the common guillemot and to evaluate the potential relationship existing between the seabirds' heath status and their contaminant loading. The birds were sampled as follows : n = 230 from the Belgian coast, n = 72 from Brittany (France) and n = 20 from the Scottish coasts (kindly provided by Prof. R.W. Furness, University of Glasgow, Scotland) (Table VII). The following discussion will be focused on the guillemots collected on the Belgian coast and a comparison will be presented with those sampled in Brittany after the Erika's oil spill and Scotland.

In particular, Zn, Pb, Ni, Cd, Fe, Cr and Cu were analyzed in the tissues (typically liver, kidneys and pectoral muscles). However Cr, Ni and Pb were most often below the detection limits and will not be further discussed.

Both teams of Forrester et al. (1997) and Daoust et al. (1998) correlating pathological and toxicological findings in common loons 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.

Material and methods.

During this research programme a total of 322 common guillemots were sampled for toxicological analysis (Table VII). A vast majority of them (n = 230) were collected dead or moribund on the Belgian coast or after a short stay (a maximum of 3 days) in a rehabilitation centre. Seventy-two individuals were sampled following the major oil spill of the tanker Erika off the Brittany coasts in December 1999 whereas the remaining 20 guillemots have been collected dead on the Scottish shores.

Origin	Condition at sampling	Sample size
Belgian coast	Beached and/or after a short stay (maximum 3 days) in a rehabilitation center.	230
Brittany, France	Heavvily oiled + beached	72
Scotland	Beached	20

Table VII : List of the different geographic origins of the common guillemots.

Forty-one percent of the guillemots 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 and van Franeker, 1992). Sex, total body weight, liver and kidney weight, as well as the presence of oil, intestinal contents, acute haemorrhagic gastro-enteropathy (AHGE) 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 and 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 analysis.

Heavy metal analysis (Cu, Zn, Fe and Cd) were performed using Atomic Absorption Spectrophotometry. Pb, Ni and Cr contents were also determined but the results most often were below the detection limits and will not be further 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. In addition, the 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 and Blackstock (1973).

Data analysis.

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 AHGE, 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.

The main characteristics of the sample analysed is reported in Table II. Most of the specimens are juveniles birds (<1 year old) of either sex. Among the different pathologies observed at necropsy, most of the individuals (93 %) presented clear signs of emaciation to varying degrees (cachectic status), a pathology characterized by a mild to severe atrophy of the pectoral muscles and complete absence of subcutaneous and/or abdominal fat deposits. 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 common guillemots had to rely on their protein reserves as an energy source prior to dying. Furthermore, the frequency of this cachectic status is not statistically correlated to neither the age, sex, wintering month considered, presence or absence of oil, or presence or absence of AHGE (all p=ns). It is worth noting that guillemots considered as non cachectic in this study have lost up to 18 % of their total body weight compared to healthier individuals weighed at colonies. Those which developed a severe cachectic status (+3) have lost up to 43 % of their total body weight (Table VIII) compared to guillemots weighed at colonies (± 1000 g.: Bédart, 1985; Harris and Wanless, 1988; Furness et al. 1994; Harris et al. 2000). 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.

	Age			Sex			Intestinal contents				Cachexia				GEAH		Oiling	
	Juv.	*Ad.*	*nd*	*Male*	*Female*	*nd*	*Norm.*	*Blood*	*Empty*	*nd*	-	*+1*	*+2*	*+3*	-	+	-	+
n	163	62	5	119	105	6	28	157	41	4	16	35	107	72	61	169	136	94
%	70.8	26.9	2.2	51.7	45.7	2.6	12.2	68.3	17.8	1.7	7	15.2	46.5	31.3	26.5	73.5	59.1	40.8

Table VIII: Sample size (n) and percentage of juvenile and adult, male and female, intestinal contents (normal, blood, empty), non cachectic (-) and cachectic with increasing severity (+1 to +3), acute hemorrhagic gastro-enteropathy (AHGE) presence (+) or absence (-) and oiling presence (+) or absence (-), of common guillemots collected on Belgian beaches from winter 1996-97 to winter 1999-2000. Not determined: n.d.

In addition, a large majority of the guillemots examined in this study (86.1 %) did not present any food remains in their stomach, whether they showed an empty stomach (17.8 %) or clear signs of an haemorrhagic content (68.3 %). Food remains were found in 28 specimens (12.2 %) and always consisted of partially digested items. In no case fresh fish pieces were recovered.

Toxicology.

A first comparison with data available in the literature (Table IX) showed that compared to guillemots captured in the Northern Norway area (Wenzel and Gabrielsen, 1994) and to those shot in northwest Scotland (Stewart et al. 1994), the individuals collected on the Belgian coast diplay high hepatic and renal Cu and Zn levels (Debacker et al. 1997, 2000).

Tissue	Sample	Time	Place	Cu	Zn	Cd	Reference
Liver	n = 83	April to Nov. 1988	Northwest Scotland	range 12.9 – 16.1	range 58.4 – 69.7	range 1.4 – 2.5	Stewart et al. 1994.
	n = 10	Summer 1992 and 1993	Hørnoya, North Norway	20.0 ± 2.9	86.7 ± 14.9	3.1 ± 1.1	Wenzel and Gabrielsen, 1994.
	n = 143	Winters 1990 to 1995.	Belgian coast (stranded)	52 ± 17	145 ± 39	2.4 ± 1.6	Debacker et al. 1997.
	n = 230	**Winters 1996 to 2000.**	**Belgian coast (stranded)**	**53 ± 22**	**164 ± 49**	**2.2 ± 1.5**	**This research program**
Kidneys	n =83	April to Nov. 1988	Northwest Scotland	range 12.3 – 15.2	range 59.3 – 74.1	range 0.8 – 3.9	Stewart et al. 1994.
	n = 10	Summer 1992 and 1993	Hørnoya, North Norway	14.4 ± 1.9	114 ± 13	1.5 ± 0.2	Wenzel and Gabrielsen, 1994.
	n = 143	Winters 1990 to 1995.	Belgian coast (stranded)	28 ± 12	169 ± 41	7.8 ± 6.6	Debacker et al. 1997.
	n = 230	**Winters 1996 to 2000.**	**Belgian coast (stranded)**	**33 ± 13**	**174 ± 42**	**7.1 ± 5.5**	**This research program**
Muscle	n = 24	April to Nov. 1988	Northwest Scotland	range 10.2 – 14.0	range 20.9 – 26.0	nd	Stewart et al. 1994.
	n = 10	Summers 1992 and 1993	Hørnoya, North Norway	19.2 ± 0.9	49.3 ± 3.3	0.2 ± 0.1	Wenzel and Gabrielsen, 1994.
	n = 143	Winters 1990	Belgian	18 ± 6	60 ± 14	< dl	Debacker et

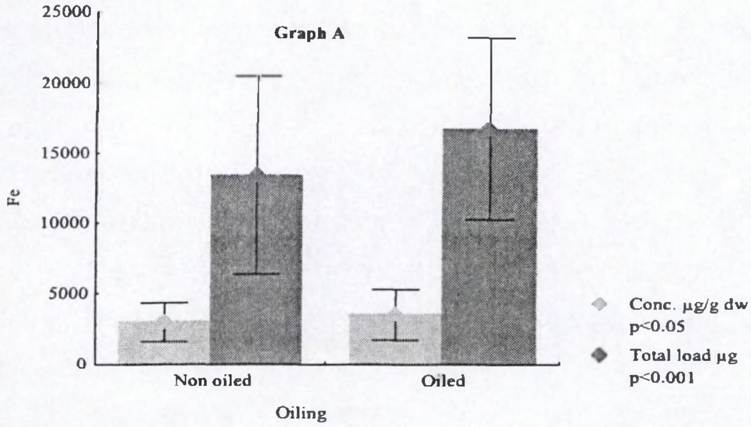
	to 1995.	coast (stranded)				al. 1997.
n = 230	**Winters 1996 to 2000.**	**Belgian coast (stranded)**	**16 $\pm$ 7**	**64 $\pm$ 21**	**< dl**	**This research program**

Table IX: Comparison of trace elements concentrations (μg/g dw), expressed as a range of mean values or as a mean $\pm$ standard deviation, in common guillemots of different origins. Datas from this research program are shown in bold. Nd: non determined, < dl: below detection limit. Adapted from Debacker et al. 1997.

Heavy metals and cachexia.

As mentionned above, a large majority of the collected guillemots presented clear signs of malnutrition characterized by the development of a mild to severe cachectic status. 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 and Morrisson, 1981; Cousins, 1985; Krämer et al. 1993; Cork et al. 1994). In wild birds, this was described by Norheim and Borch-Iohnsen (1990) for the common eider, by Wenzel and Adelung (1996) for the common guillemot and also by Esselink et al. (1995) for a land-based species, the barn owl. In a previous study, 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. 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 AHGE was made. It was found that Cu and Zn concentrations in both liver and kidneys but also Zn in muscles are highly dependent on the cachectic status. 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; Wenzel and 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 AHGE 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.

Compared to concentrations, the cachectic status of the animal is of little influence on the organs' total metal loads 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 (Figure 14) 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 and herring gull (Leighton et al. 1983) but also for the common guillemot (Fry and Lowenstine, 1985; Khan and Ryan, 1991). However, in the case of stranded birds, post mortem oiling of the carcass cannot be ruled out.



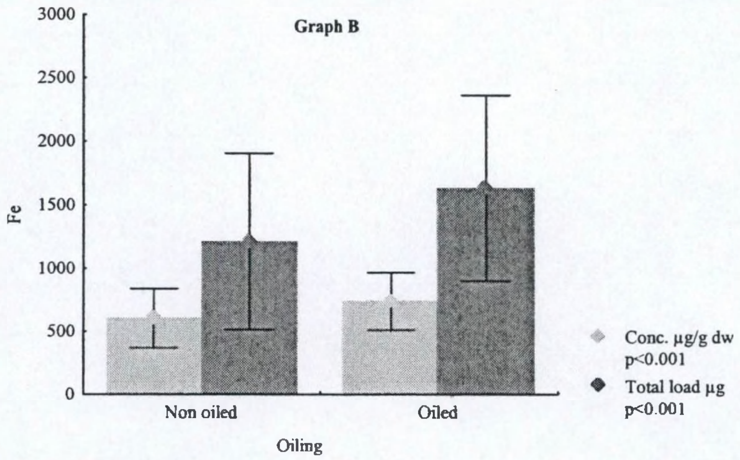


Figure 14: Hepatic (graph A) and renal (graph B) iron concentration ($\mu g\ g^{-1}$ dw) and total load (μg) of common guillemots.

Comparison with guillemots sampled in Brittany after the Erika's oil spill.

After the Erika's oil spill off the coast of Brittany, France and following close contacts with the French parties, the Belgian MARIN group was asked to carry out necropsies and sampling on approximatively 100 heavily oiled birds, mainly common guillemots. Seventy-two were made available for toxicological investigations.

A condition index was calculated using the liver to kidney weight ratio, as proposed by Wenzel and 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. Guillemots with a condition index ≤ 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.

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 X, XI and XII).

		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 X : Trace element 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 X), while hepatic Cu and Zn levels remain similar between the two 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). Common 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 (Baillie 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 guillemot 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 and Adelung, 1996) and potential contamination of these adult females while breeding in the vicinity of more polluted sites cannot be excluded.

		Condition index $2 < x \leq 2.5$			
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 XI : 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), among which a few individuals with higher hepatic and renal Cd concentrations were found. 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 XII : 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 XII).

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, 1993a ; 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 and Adelung (1996) comparing guillemots collected stranded or moribund at the German Bight with individuals collected in Northern Brittany (Channel area). 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.

Comparison with guillemots sampled stranded on the Scottish coast.

A total of n = 20 common guillemots collected in Scotland were made available for toxicological analysis. A large majority (90 %) of these animals were cachectic and among them 1/3 were severely emaciated.

Similarly, Cu, Zn and Fe concentrations in the livers, kidneys and pectoral muscles were comparable to those described in the Belgian guillemots. However, probably due to a small sample size, increasing concentrations of metals could not be detected in parallel to increasing cachexia severity. In contrast, Cd concentrations in the three organs were clearly much higher than those described for the Belgian sample and also showed a strong age-dependent distribution (Figure 15).

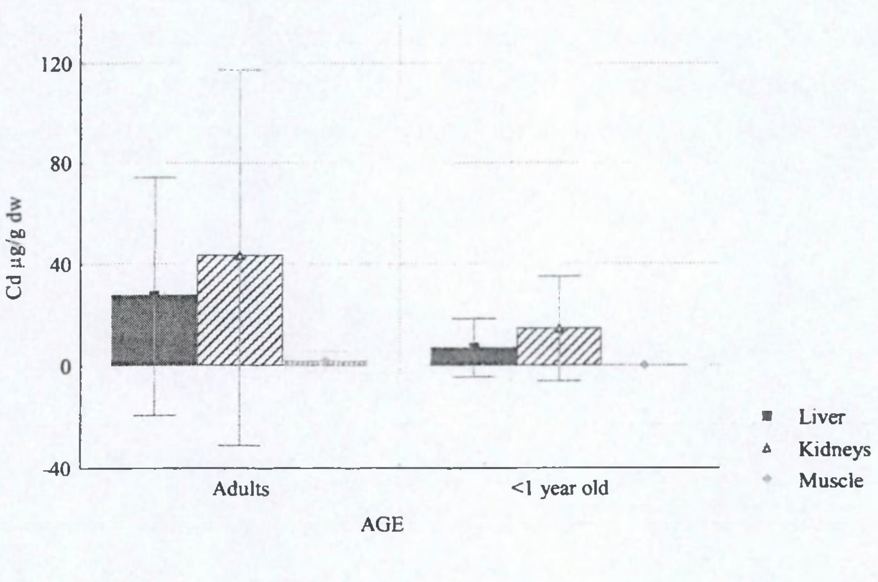


Figure 15 : Distribution of Cd with age in the livers, kidneys and pectoral muscles of guillemots collected in Scotland.

Conclusions.

Heavy metal 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 and 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 common guillemots and could well represent an additional source of stress to birds already facing stressful conditions.

When compared to guillemots sampled in Brittany, 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 common 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.

Metallothioneins (adapted from Debacker et al. 2001a).

Introduction.

Metallothioneins (MTs) are widely recognized as playing a key role in metal detoxification and homeostasis. Once bound to MTs the potential toxic effects of the metals appears to be buffered, underlying the importance of the study the speciation of these metals. MTs analysis in free living seabird species often demonstrated strong positive correlation between the MTs contents of the organs and its heavy metal levels (renal MTs and Cd concentrations and/or hepatic MTs and Cu and Zn concentrations) (Hutton, 1981 ; Cosson, 1989 ; Elliott et al. 1992 ; Stewart et al. 1996 ; Elliott and Scheuhammer, 1997). In a previous study, Bouquegneau et al. (1996) demonstrated that most of, but by far not all, the additional copper ions were bound to cytosolic MTs, probably as a result of the great affinity of the metal for MT compared to cadmium and zinc.. Increasing Cu and Zn concentrations were clearly linked to an increasing cachectic status for guillemots collected at the Belgian coast (Debacker et al. 1997, 2000a). 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 and Adelung (1996) for common guillemots collected in the more polluted German Bight. 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 and 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.

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 26,000 g. 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. After adding nitric acid 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. 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 6,800 Da typically binding 7 bivalent metal ions (Hamer, 1986; Kägi, 1993; Kojima et al. 1999).

Results and discussion.

Metallothioneins 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 ≤ 2 , and those – more robust - with a condition index > 2, as suggested by Wenzel and 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 (Table XIII). In our study, the liver of those emaciated guillemots had significantly lost weight compared to more robust specimens.

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 ↗ ↗
Cu-MT / 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 XIII: 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: ↘ : $p < 0.05$; ↘ ↘ : $p < 0.01$; ↘ ↘ ↘ : $p < 0.001$).

Metallothioneins and heavy metals.

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 degrading body condition, while the percentage of Zn simultaneously decreased (Figure 16).

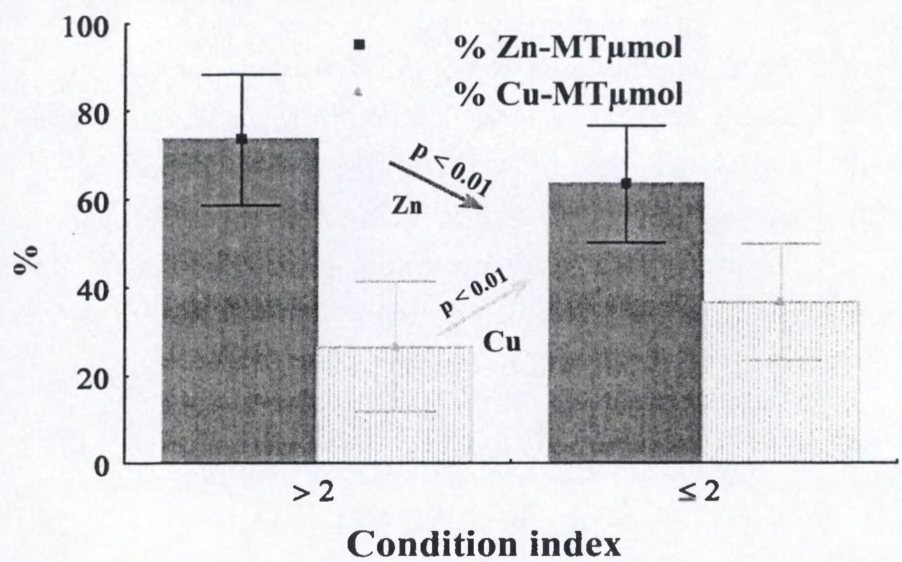


Figure 16: Relative distribution of Cu and Zn (%) when stored on MTs in relation to the individuals' condition index.

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). 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 17) 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.

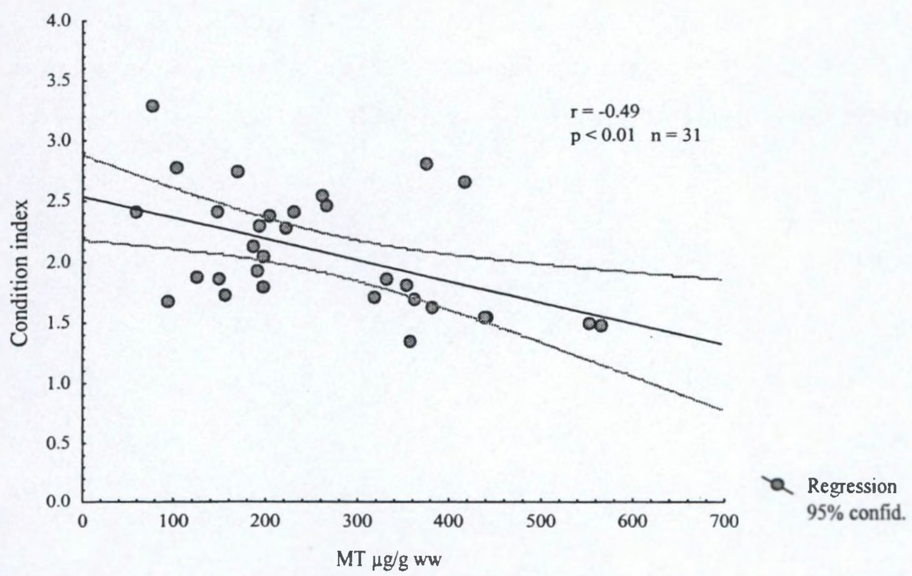


Figure 17: Condition index versus MT concentration in the livers.

A rather different situation appearedfor Cd, for which the age, 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.

Conclusions.

To conclude, MTs appeared to be involved in the physiological response to the general heavy metals homeostasis disruption induced in the emaciated common 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 common guillemots displayed higher MT as well as Cu- and Zn-MT concentrations.

Experimental contamination **(adapted from Debacker et al. 2001b).**

Introduction.

A large majority of the common guillemots found stranded at the Belgian coast over the past few years presented evidence of malnutrition characterized by a cachectic status (Jauniaux et al. 1996,1998). A clear relationship was established between this cachectic status and both the heavy metals distribution and their concentration in target organs (livers and kidneys). In particular, increasing levels of Cu and Zn were detected with increasing cachexia severity (Debacker et al. 1997, 2000).

With such findings the question inevitably arises 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 sets of experiments, using the common quail, as an experimental model, were carried out. 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).Combined effects of heavy metals contamination (Cu, Zn and CH_3Hg) and starvation were tested on the experimental animals which were used as a model for comparison with the wild common guillemots 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.

Material and methods.

Animals.

Seven week old male quails 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 0.01g 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.

Contamination with Cu, Zn and Hg at a given concentration, followed by a 4-day complete starvation.

During this 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.

See procedure p. 48.

Heavy metals analysis.

See procedure p. 48-49 (Zn, Pb, Ni, Fe, Cd, Cr and Cu).

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

Data analysis.

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 and discussion.

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), 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).

Conclusions.

To conclude, the experimental contamination tests using the common quail was considered to be a useful tool for comparison with a wild population of common guillemots. The causes of the severe emaciation symptoms 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 levels encountered could well, in turn, be active participants favouring a generalized lessened body condition.

Seabirds : General discussion and conclusions.

Stranded common guillemots collected at our coasts most likely represent only a small fraction of the total number of dead birds at sea during the winter as it is a known fact that not all dying birds at sea are recovered on beaches (Bibby and Lloyd, 1977; Bibby, 1981; Bodkin and Jameson, 1991; Hlady and Burger, 1993; Van Pelt and Piatt, 1995; Piatt and Ford, 1996; Flint and Fowler, 1998). To evaluate the chance of recovery of the corpses, drifting experiments were carried off the Belgian coast. Results indicated a strong influence of the wind climate (direction and velocity) in the days after the dropping: the closer to the coast the corpses are released, the higher the probability that they will wash ashore. In addition, persistence experiments demonstrated that a bird corpse remains on the beach for 5.8 – 13.3 days, with the minimal value probably being the more realistic one (Seys, submitted c).

However, a comparison with the population found wintering in the Southern North sea is difficult as an 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.

Stranded specimens presented evident signs of a progressive debilitation and were recovered in a generally very poor health status. At necropsy, among other findings, a large majority of these common 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). Similar observations are reported in the literature as a striking feature not only of stranded seabirds (Parslow and Jefferies, 1973 ; Hope Jones et al. 1984; Camphuysen, 1989; Cunningham and Simmonds, 1992; Forrester et al. 1997; Piatt and Van Pelt, 1997; Daoust et al. 1998), but also of land based species (Esselink et al. 1995 ; Thouzeau et al. 1999a, 1999b). 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 common guillemots had to rely on their protein reserves as an energy source prior to dying.

In addition, a large majority of the common guillemots examined in this study did not present any food remains in their stomach. They they showed an empty stomach or clear signs of an haemorrhagic content. Although it is not surprising, it is worth noting that most of these individuals were also cachectic. Food remains were found in a very few individuals and always consisted of partially digested items. In no case fresh fish pieces were recovered. Intestinal dysfunction (altered epithelial transport) has been shown to increase in malnourished rats (Darmon et al. 1993). Such changes might well have negative effects on the digestive tract's integrity (favouring invading pathogens) and digestive function (Klasing, 1998).

Evidence of pollutants 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 pollutants 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 and organochlorines as additional debilitating agents in a population of wintering common guillemots found stranded at the Belgian coast.

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. These differences became even more striking when taking into account the different categories attributed to the cachectic status: 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 acute hemorrhagic gastroenteropathy - AHGE - 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.

In response to combined environmental stressors (e.g. cold weather, starvation, oiling..), the common guillemot's body and organs weight were shown to significantly decrease in cachectic individuals. In particular, the livers lose much more weight than the kidneys whereas 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 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 on the age of the individuals, with the highest levels observed in the kidneys of adult common guillemots. 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 and Furness, 1998). In our study such a distinction could not be made as the birds were aged following the presence of the cloacal bursa with no idea of the precise age. However, Cd levels detected in the stranded common guillemots are well below the proposed hepatic and renal Cd treshold levels above which Cd poisoning might be expected (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 and Adelung (1996). As expected, the condition index and cachectic status were negatively correlated, with lowest condition index observed for severely cachectic individuals.

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 and total Hg. 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 common 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 common guillemots collected in Brittany (France), following Erika's oil spill to those collected at the Belgian coast. As body condition was shown to drastically influence heavy metal levels in the tissues, we compared common guillemots of similar condition index and invariably found higher levels of Cu and Zn in all three tissues of the Belgian common guillemots. This holds true even when comparing the more robust common guillemots of both origins, suggesting that contaminant levels tend to be higher in the Southern North Sea.

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 common guillemot's general health status.

To answer this question, a set of experiments using the common quail was developed to test the combined effect of contamination (using Cu, Zn and CH_3Hg) and starvation. Appropriate heavy metal levels were given to the quails to obtain concentrations similar to those found in the common 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 (MTs) - 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 common 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. 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 and Brown, 1985). In the case of the guillemots collected stranded on the Belgian coast, we can not exclude periods of partial feeding prior to 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 common 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.

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 common guillemots could well be adversely affected by their contaminant levels (Cu and Zn) with degrading body condition.

MARINE MAMMALS

Technical aspects of strandings of marine mammals: Interventions and field problems.

Introduction.

The immediate response in case of marine mammal strandings needs a good measure of co-ordination, and sometimes – especially in the case of strandings of whales – the intervention of many different services and people. The response and the necessary equipment differ on a case by case basis. During the first years of dealing with strandings, often much improvisation was needed. It is clear that responsible authorities are better prepared now, and improvisation can be kept to a minimum.

Informing the relevant authorities and recording sightings.

Getting informed about strandings of marine mammals is of course the first, and very important step. For getting informed, the RBINS establishes contacts with local authorities along the coast (fire departments, police), with volunteers, fishermen, aquaria (Sea Life Blankenberge and Aquarium Oostende), the Marien Ecologisch Centrum (MEC, Oostende), etc. The experience and assistance of veterinarian surgeon Dr. John Van Gompel is very valuable.

The RBINS regularly receives reports of sightings of marine mammals from sailors, other people that spend time at sea, and scientists on bird survey campaigns. On some occasions, corpses of marine mammals found dead at sea or in fishing gear (bycatch) are received from fishermen. Fishermen are encouraged to deliver dead marine mammals and seabirds (instead of throwing them back into the sea), but do not receive a reward for doing this. Bycaught animals are very valuable for research purposes. Basic strandings data and data of bycatches and sightings are collected in a database held by the RBINS (MUMM Oostende).

Available equipment in case of strandings.

During the last years, gradually more and more useful equipment for intervening in case of strandings became available. At the RBINS (MUMM Oostende) the following equipment is standby and can be deployed (not complete):

- Mobile phones;
- Van (Volkswagen Transporter);
- Transport stretchers (one for dolphins, one for small dolphins or porpoises)
- Water containers (180 l), and a waterpump with shower (12 V);
- Medical kit (with Valium, Solu-Delta-Cortef, disinfectant, etc.);
- Small equipment: disposable rubber gloves, body bags, measuring tape, etc.;
- Video camera (digital), photo camera (35 mm);
- Freezer.

Sometimes small problems occurred because no off-road vehicle was available. In those cases, and in cases of larger marine mammals strandings, the assistance of fire department, police or Civil Protection was invaluable. On one occasion the RIB (Rigid Inflatable Boat) based at the RBINS (MUMM Oostende) was used to recover a dead dolphin at sea (the carcass turned out to be too decomposed to be returned to port).

Response to strandings by the RBINS.

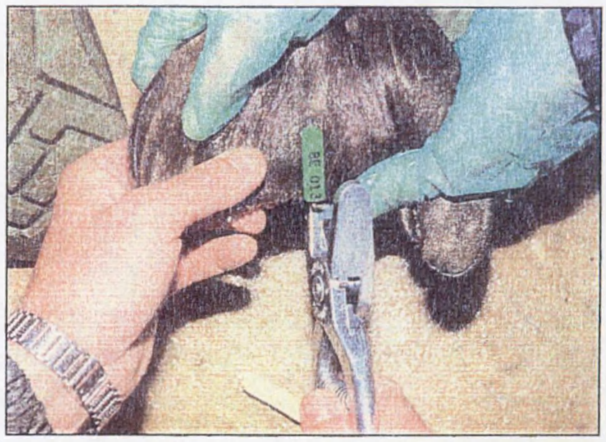
The response after a report of a marine mammal stranding is different according to the type of stranding. We can make the distinction between strandings of live seals, live small cetaceans, dead seals or small cetaceans, and larger cetaceans.

Strandings of live seals.

Live seals (wounded, sick, pup) are dealt with by Sea Life Blankenberge, where since 1998 a seal rehabilitation centre exists. Reports of live seals taken up at Sea Life are immediately passed on to the RBINS. Up to spring 2001, 25 common seals were rehabilitated at this centre, and subsequently released in the Western Scheldt. Also 13 grey seals were rehabilitated, and released at Knokke-Heist. One hooded seal was released in the North Sea, with assistance of the Belgian Navy (Haelters, 2000a; Haelters and Kerckhof, 2000). For the release of seals, Sea Life requires the authorisation of the RBINS (MUMM) and the Flemish and Dutch (for harbour seals) authorities.

In order to recognise individual seals, they are tagged and are inserted a subcutaneous transponder (*INDEXEL*), provided by the RBINS. The green tag has a number, preceded by "BE", and the inscription MUS.SC.NAT.BRUSSELS (see Picture 1).

Picture 1: Tagging of individuals for identification.



Strandings of live small cetaceans.

Between 1996 and July 2001, 4 live cetaceans were transported to the Marine Mammal Park at Harderwijk, The Netherlands: a harbour porpoise, a striped dolphin, a white-beaked dolphin and a white-sided dolphin. The striped dolphin stranded near Boulogne (France), and was transported at the request of the aquarium Nausicaa (Picture 2). Small animals are only transported mid-way between the Belgian coast and Harderwijk. All animals reached Harderwijk alive, but died shortly after their arrival. Trying to save live stranded cetaceans is worth the effort. First of all it is important to understand that the public expects a rescue operation. The most important goal is to learn more about the animals during their rehabilitation, which can be beneficial to the whole species (Read et al. 1997).

Strandings of dead seals or dead small cetaceans.

Dealing with small cetaceans or seals is usually technically not too difficult. Sometimes the assistance of local authorities is needed. Because carcasses are often decayed, rubber gloves and body bags have to be used. Very decayed small animals are intermediately stored in the freezer at Oostende. Fresh animals, or animals which are too large for freezing, are immediately taken to the University of Liège. Sometimes this is done with the assistance of the Civil Protection based at Jabbeke near Oostende.

Larger numbers of stranded marine mammals mean an increased effort. While in the early 1990s only 3 to 6 harbour porpoises washed ashore in Belgium, this increased to between 8 and (over) 18 per year . The growing number of strandings of harbour porpoises, and to a lesser extent seals, is not necessarily the result of a better reporting (Haelters et al. 2000). Also at sea, higher numbers of harbour porpoises are seen (fishermen, personal communications; unpublished data from the database held at MUMM; recent sightings reported in De Ridder, 2001). In Dutch waters, an increase in sightings was reported by Camphuysen and Leopold (1993), Camphuysen (1994) and Witte et al. (1998). Seals also seem to have become more common recently.

It is clear (from sightings and strandings data) that more porpoises are present in Belgian waters from February until July. The calves are born around June, and especially during the last years, some neonates, and even stillborn calves washed ashore. Also for the first time, a pregnant female washed ashore (2001). This indicates that the Southern North Sea is used as an area of reproduction.

Dealing with large cetaceans.

Picture 3

The stranding of one or more large cetaceans is an unusual event. Due to this, and due to the fact that every stranding is different, a certain level of improvisation and ad hoc consultation with different authorities is necessary. In Belgium, only 1 whale stranded during this project: a juvenile fin whale on 1 November 1997 (Picture 3). However, the intervention network assisted in the autopsies and scientific research of a couple of whales stranded in the north of France and the Netherlands.

The larger and the more decayed a cetacean, the more technical difficulties one encounters. A co-operation with third parties is needed for technical assistance (Tavernier, 1997) and for dealing with the public (management of crowds and their safety). Because of the sometimes large amounts of decaying waste, responsible authorities for waste treatment are also faced with technical and environmental problems. Necropsies usually cannot start before a solution has been found for these problems. The press needs to be informed on a regular basis.

Compared with the past, scientific research on stranded whales clearly has improved. In September 1984 a fin whale was pulled into the harbour of Nieuwpoort. This animal was buried, without being properly investigated or sampled. In 1989, a sperm whale was buried a couple of days after stranding, and only few samples were taken. The 4 sperm whales that stranded in 1994 however were thoroughly investigated (jacques, 1997; Jacques and Lambertsen, 1997), which can also be said of the fin whale that stranded in 1997.

The intervention network has gained a great deal of experience in dealing with larger marine mammals during the last years, also because of the co-operation that was established with neighbouring countries.

The importance of informing the public.

In order to be informed about strandings, it is important to regularly inform local authorities and the public. To achieve this, the RBINS regularly updates information in letters to local authorities. A small booklet with information on how to react to strandings or bycatches was published by the RBINS (MUMM ; Haelters, 1997), and distributed to local authorities, fishermen, local nature conservation organisations, etc. The RBINS (MUMM) also prepared two leaflets (1999 and 2001) with information about marine mammals, and what to do in case of strandings. The first leaflet was prepared together with the Flemish Community and two NGO's (AMINAL et al. 1999), the second together with Sea Life (see above : front page). Both leaflets were distributed in large numbers at the coast. On a regular basis the network was promoted in local magazines (Haelters, 1999a , 1999b ; Haelters, 2000b ; Tahon and Haelters, 1998).

Conclusions.

During these last years a lot of experience has been gained due to the larger number of marine mammals stranding and by a better co-ordination of all authorities involved. Also better equipment was available, including better communication means. The work was also facilitated by a growing support of the public, and the excellent co-operation with local authorities, volunteers, Sea Life Blankenberge, the Marien Ecologisch Centrum, the Civil Protection, etc. Difficulties were sometimes encountered because of not having an off-road vehicle at our disposal.

Marine mammals necropsies and causes of death.

The most frequent causes of death of North Sea marine mammals are bycatches in fishing gear and acute bronchopneumonia (Baker and Martin, 1992; Kuiken et al. 1994; Kirkwood et al. 1997), entanglement in fishing nets being pointed at as a serious threat to some cetacean populations.

Morbillivirus infections (PDV infection) among marine mammals appeared for the first time in the North Sea in 1988 and were responsible for the death of many thousands of harbour seals and hundreds of grey seals in 1988 and 1989 (Kennedy et al. 1988; Osterhaus et al. 1988; Kennedy, 1999). Since, no cases had been reported in North Sea seals, but serological studies suggested that PDV infection had persisted in this area (Visser et al. 1993). In the early 1990s, a few cases of morbillivirus infections were described in porpoises (Kennedy et al. 1991, 1992). The occurrence of morbillivirus associated diseases in large cetaceans, and particularly in baleen whales, had not been documented to this day.

Material and method.

Collection of animals.

Increased efforts have been made to collect stranded or bycaught marine mammals along the Belgian coast and from Northern France, from the Belgian border to the Bay of the Somme (Table XIV). Carcasses were forwarded to the Department of Veterinary Pathology of the University of Liege. Large cetaceans were necropsied on the beaches.

Cetaceans	Harbour porpoise	*Phocoena phocoena*	77
	White-beaked dolphin	*Lagenorhynchus albirostris*	8
	Fin whale	*Balaenoptera physalus*	4
	White-sided dolphins	*Leucopterus acutus*	2
	Striped dolphin	*Stenella coeruleoalba*	2
	Sowerby's beaked whale	*Mesoplodon bidens*	1
		Total for cetaceans	**94**
Pinnipeds	harbour seal	*Phoca vitulina*	36
	Grey seal	*Halichoerus grypus*	7
	Hooded seal	*Cystophora cristata*	1
		Total for pinnipeds	**44**
		TOTAL	**138**

Table XIV : Marine mammals necropsied between 1997 and 2001.

Necropsies and samplings.

Standard protocols were used for small cetaceans (Kuiken and García Hartmann, 1991), seals (Geraci and Lounsbury, 1993) and large cetaceans (Jauniaux et al. 1999). Briefly, animals were measured, weighed and blubber thickness was measured at the caudal insertion of the dorsal fin. After external examination, the abdominal and thoracic cavities were opened and the skull was longitudinally sawed for central nervous system observation.

For histopathology, samples of eye, skin, liver, lymph nodes, gonad, reproductive tract, oesophagus, stomach, intestine, kidney, urinary bladder, pancreas, lung, heart, thyroid, thymus and brain and tissues with lesions were fixed in 10 % buffered formalin, embedded in paraffin. Sections (5 μm) were cut and stained with haematoxylin and eosin (HE). Selected sections were also stained with Masson trichrome, periodic acid Shiff (PAS) for fungi and Ziehl-Neelsen for acid-fast organisms. In addition, immunohistochemistry using a monoclonal antibody against the glycosylated haemagglutinin protein of phocine distemper virus (clone 1.3) (Trudgett et al. 1991; Domingo et al. 1992) was performed on lungs, pulmonary lymph nodes and lesions. The other reagents used in this technique were the Enhanced Polymer One-Step Staining procedure commercial kit (Dako Envision™, Dako, Denmark) (Jauniaux et al. 2000).

For bacteriology, tissue samples were collected aseptically and incubated under aerobic and anaerobic conditions on Columbia blood agar with 5 % sheep blood (Becton Dickinson Benelux s.a., Belgium) and on a selective medium for *Enterobacteriaceae* (Gassner agar, Merck, Merck-Belgolabo s.a., Belgium).

Blood samples were collected and examined for the presence of *Brucella abortus* (Tryland et al. 1999) and canine distemper virus (CDV) (Appel and Robson, 1973).

Parasites were collected and preserved in 70 % ethanol with 5 % glycerin.

In suspected cases of viral disease, Vero cell cultures were infected as previously described for seals (Jauniaux et al. 2001).

The harbour porpoise was used as reference animal to describe lesions and causes of death among cetaceans while the harbour seal was referenced for pinnipeds.

Results.

Small cetaceans : the harbour porpoise.

Thirty one females (one pregnant) and 41 males (sex not determined in 5 case) were examined, distributed in 6 neonates, 43 immatures and 21 matures (age not determined in 7 cases). The annual distribution was very irregular with major rises in 1999 and 2001 (Figure 18). Most strandings occurred during winter and early spring.

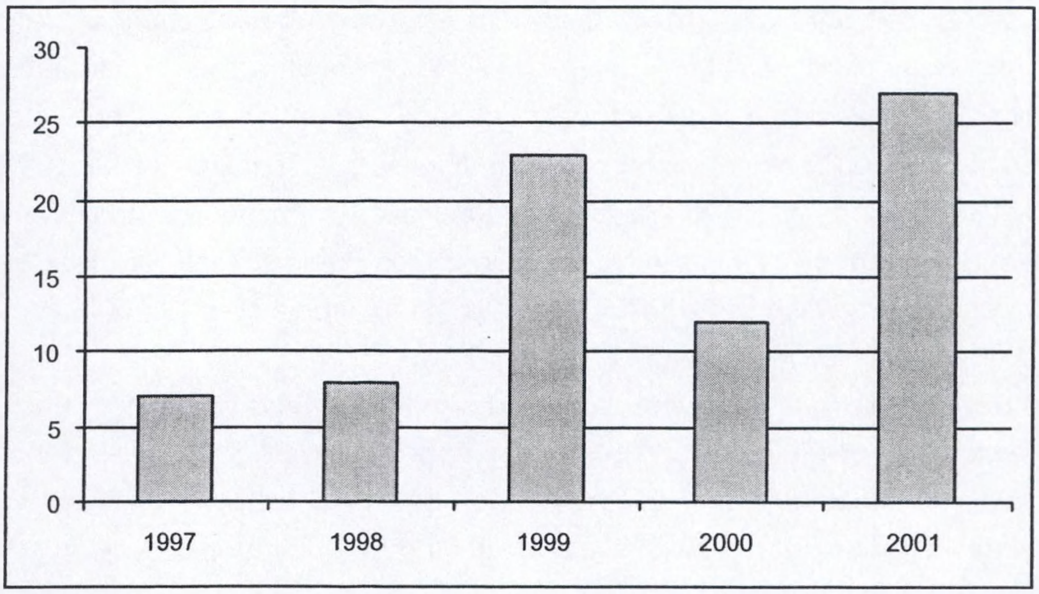


Figure 18: Annual numbers of porpoises necropsied from the considered area from 1997 to 2001.

Gross pathology.

The most common findings were severe emaciation (60 %), acute bronchopneumonia (49 %), and extended and multisystemic parasitosis (51 %). It was usual to find more than one apparent cause of death in one porpoise, fatal conditions being closely interwoven.

Body condition.

Severe emaciation was characterized by weight loss, reduced blubber layer thickness and dorsal muscle atrophy. Mean blubber thickness for emaciated porpoises was 9,2 $\pm$ 3,8 mm and 20,1 $\pm$ 10,7 mm for non-emaciated porpoises ($p < 0,0001$). Blubber thickness was not associated with age and/or sex (data not shown).

Bronchopneumonia and other intra-thoracic findings.

Acute bronchopneumonia was characterized by large areas of lung consolidation with haemorrhagic and/or purulent fluid oozing from the lung parenchyma. Most often, it was associated with massive *Torynurus convolutus* and *Pseudalius inflexus* infestation in airways, the latter parasite being firmly attached to the lung parenchyma. Small airways were frequently partially or completed occluded. Pneumonia was associated with *Escherichia coli* ($n=2$), a non-haemolytic *Staphylococcus* sp. ($n=3$), a haemolytic *Streptococcus* sp. ($n=3$), *Aeromonas hydrophyla* ($n=1$) and *Proteus vulgaris* ($n=1$) ; all cases of *Pseudomonas* sp. infection ($n=4$) were associated with an acute necrotizing pneumonia. Parasitic infestation was also present in all of these cases of bacterial infection.

Of the 6 neonates, 2 were considered to be stillborn since the lungs were entirely atelectatic. Another was regarded as an aborted foetus because an 82 cm long umbilical cord remained attached. Three porpoises had mild parasitic infestation of the pulmonary airways without evidence of bronchopneumonia.

Severe and extended parasitosis.

The second most frequent observation was severe and extended parasitosis consisting of heavy infestations of multiple organs with associated lesions of bronchopneumonia, chronic ulcerative gastritis and chronic hepatitis (Table XV).

Species	**Organ**	**Prevalence in parasited porpoises**	**Associated lesion**
NEMATODES :			
Pseudalius inflexus	Bronchi, blood vessels and heart,	63,8%	Bronchopneumonia
Torynurus convolutus	Bronchi	68%	Bronchopneumonia
Halocercus invaginatus	Lung parenchyma	9,5%	Bronchopneumonia
Stenurus minor	Middle ear	55,3%	No lesion
Anisakis simplex	Stomac	57,5%	Chronic ulcerative gastritis
TREMATODES :			
Campula oblonga	Bile and pancreatic ducts	31,9%	Liver fibrosis
Pholeter gastrophilus	2^{nd} gastric compartment	17,8%	Nodular gastritis
CESTODES :			
Diphyllobothrium stemmacephalum	Intestine	8,5%	Enteritis

Table XV: Parasites location and associated lesions among porpoises stranded among the Belgian and northern French coastline.

Bycatch.

Bycatch in fishing nets was considered to be the cause of death of 20 % of porpoises. Three animals were known bycatches, while others were suspected of having been thrown back into the water after capture. All of these animals were in good nutritional condition, without evidence of severe disease. However, they all had extensive lung congestion and oedema, abundant white froth within airways which was frequently blood-tinged, subcutaneous haemorrhage and peri-mortem skin incisions. One suspected bycatch turned out to have been trapped after death. It showed post-mortem skin lacerations, a thin blubber layer (8 mm) and evidence of an acute bronchopneumonia.

Other findings.

Acute to chronic ulcers were observed on the oesophagus of 8 porpoises. Ulcers were usually localised around the larynx or along the cranial third of the oesophagus. Acute ulcerative stomatitis was seen in 6 cases. Numerous round or elongated acute ulcers were present on the palate, tongue or gingiva; some were covered with a fibrinous membrane. Acute enteritis, diagnosed in 5 porpoises, was associated with hyperaemia of the serosal membrane and intraluminal haemorrhagic exudate. One porpoise showed signs of pyaemia, with abscesses distributed throughout the lung, liver, kidney and myocardium. *Streptococcus equisimilis* was isolated from the blood and lesions.

The gastro-intestinal tract was empty, without evidence of recent feeding, in 40 animals and contained fresh preys or otoliths in 8 cases (not examined in 7 cases). Some animals presented mild parasitic infestation of the gastric cavity (6 cases), intestines (4 cases) and bile ducts (2 cases) without associated lesions.

Histopathology.

Respiratory lesions.

In the lung, 3 major patterns of reaction were observed microscopically. Interstitial pneumonia was characterised by peribronchial lymphocytic infiltration and thickening of interalveolar septa by macrophages, lymphocytes and occasional eosinophils. This first pattern of reaction was usually associated with adult nematodes in airways. The second type of reaction was a more acute, lesion being characterised by neutrophilic infiltration in the parenchyma, and by exudation of fibrin, neutrophils, and erythrocytes into the airways. Haemorrhages, emphysema and atelectasis were frequently present. In severe cases, large areas of parenchymal necrosis were observed with loss of alveolar epithelium and only alveolar septa remaining visible. The third pattern of lesion was associated with net entrapment. In these cases, the lung was severely congested, and pink oedema fluid, containing erythrocytes, was present within the parenchyma, giving a solid pattern to the lung tissue.

Muco-cutaneous lesions.

Muco-cutaneous ulcers were mostly subacute to chronic and associated with infiltration of macrophages and fibroblasts in the underlying subcutis. Mitotic figures were numerous in the *stratum basale* around ulcers. Some tongue ulcers extended to the muscle resulting in mild myositis or muscle fibre degeneration. Few inflammatory cells were associated with esophagus ulcers, and no evidence of inclusion bodies was observed. PAS staining was negative in all cases.

Nervous and eye lesions.

Various inflammatory reactions were observed in the central nervous system of 6 porpoises. One case of severe subacute granulomatous encephalitis, affecting all the cerebral areas, was characterised by perivascular cuffings of macrophages and lymphocytes and large foci of haemorrhage. Areas of cerebral necrosis were massively infiltrated with macrophages. Viral-like eosinophilic intranuclear inclusion bodies were distributed throughout these lesions. One case of subacute meningoencephalitis was characterized by severe perivascular cuffings with macrophages and a few lymphocytes. A few trematode-like eggs with thick yellow, birefringent walls were associated with this lesion. Three animals had a mild focal subacute encephalitis with local perivascular cuffing in the parietal or occipital cortex and, in 2 of these cases, small necrotic foci were infiltrated by macrophages. Finally, the animal dead of pyaemia had small abscesses distributed throughout the brain. Perivascular cuffs of lymphocytes, plasmacytes and eosinophils were observed under the retina of 2 animals and subretinal haemorrhage was observed in 2 porpoises with hyphaema. Neither immunohistochemistry, nor cell cultures revealed evidence of morbillivirus infection or other viral disease. Serology was negative for *Brucella abortus* in all tested porpoises.

Pinnipeds : the harbour seal.

Animal collection.

There were 26 juveniles and 6 adults (age not estimated in 4 cases) distributed as 18 males and 13 females (sex not identified in 5 cases). Fresh carcasses were necropsied a few hours after death or stranding. Other animals were in various stages of preservation and 14 were only partially necropsied due to advanced decomposition.

Gross pathology.

The main findings were emaciation with reduction or absence of fat and muscles atrophy, acute pneumonia and enteritis.

Body condition.

Eleven seals were emaciated, with severe subcutaneous fat reduction and extended amyotrophy. The dorsal fat thickness for common seal varied following nutritional condition (emaciated:3,9 mm $\pm$ 4,8 ; non-emaciated: 23,2 mm $\pm$ 6,8 ; $p < 0,0001$), mortality process (traumatic death including bycatch: 23,0 mm $\pm$ 7,7; non-traumatic death: 7,0 mm $\pm$ 8,9; $p = 0,001$) and age (juvenile: 8,5 mm $\pm$ 9,2; adult: 22,0 mm $\pm$ 11,1; $p = 0,016$).

Respiratory system.

In case of pneumonia, lungs were congested with irregular surface and interstitial and subpleural emphysema. Sections revealed patches of red and/or gray consolidation. Frothy fluid, containing blood or pus in some cases, was observed in the airways. Most frequently, cranial pulmonary lobes were most affected. In cases of by catch in fishing nets (confirmed or suspected) animals had extensive lung congestion and edema with abundant white froth within airways frequently blood-tinged and subcutaneous hemorrhages. Nematodes were observed in bronchi (five seals) and in the pulmonary artery (two seals) reaching the right ventricule in one case. One animal had bilateral acute pleuritis with fibrinopurulent exudate in the thoracic cavity and numerous chronic lung abscesses.

Digestive system.

In 6 animals, acute enteritis was characterized by an haemorrhagic intestinal content, and an enlarged oedematous mesenteric lymph nodes. One seal had severe acute peritonitis associated with traumatic gastric perforation by a foreign body (metallic piece, 14 cm length, used on fishing lines), one triangular part being in the stomach, the straight extremity in the abdominal cavity. In dead stranded seals, the gastrointestinal tract was always empty, without evidence of recent feeding.

Myoskeletal system.

In 3 seals, numerous muscle hematomas were present on the head and the thorax and in 2 cases, multiple rib fractures and hemothorax were associated. One of these seals was crushed by a motorbike when it was laying on the beach while the origin of the trauma for the 2 others was not determined.

Histopathology.

Respiratory system.

Acute broncho-pneumonia was characterised by exudate of neutrophils and large macrophages, in alveoli and bronchioli associated with parenchymal necrosis areas. Interstitial pneumonia, observed on three seals, was characterised by thickening of interalveolar septa by macrophages, lymphocytes and edema, with in one case, few giant cells. In case of nematodes infestation, eosinophils were also present in the interstitial tissue with peribronchial lymphocyte infiltration. *S. phocae* and *A. hydrophila* were isolated from lungs of a seal with acute pneumonia.

Digestive system.

In three seals, subacute gastritis was characterized by eosinophils and giant cells infiltration in the submucosa. In the most severe case, the gastric wall was thickened and formed an ulcerated nodule. In the submucosa, eosinophils granulomas, some containing nematode larvae, were limited by giant cells. Subacute enteritis was observed in 4 cases, with mild infiltration by eosinophils and few lymphocytes in intestinal villi. No parasite was observed in the lumen of the intestine.

Lymphoid tissue.

In mesenteric and gastric lymph nodes of 3 seals, numerous granulomas composed of eosinophils were disseminated in lymphoid tissue. Frequently, macrophages, giant cells and fibroblasts surrounded the granuloma. Giant cells and few fibroblasts were also frequently disposed around homogenous and amorphous eosinophilic matrix positive with P.A.S. staining characterizing Splendore-Hoeppli reaction. Cytoplasm of some giant cells stained also positively with P.A.S. In the center of some granuloma, nematode larva was present but was not systematically observed. In one mesenteric lymph node, nematode larvae were observed in the lymphoid tissue. In one seal, few giant cells were present in the thymus without evidence of other lesions. Most of the time, lymphoid tissue appeared to be depleted.

Morbillivirus detection.

The results of investigations for morbillivirus infection are listed in Table XVI. Seven seals were infected by a morbillivirus during the 1998 summer (Jauniaux et al. 2001), and five other cases were positive by immunohistochemistry (n = 2) or RT-PCR (n = 5). On one seal stranded in 1999, heavy immunofluorescence was detected in the lung, typical paramyxovirus viral particles were observed by negative staining from lung tissue, and intracytoplasmic specific labelling was detected in lymphocytes of the spleen using PDV 1.3 Mab. Heavy intracytoplasmic and intranuclear labelling were also detected in lymphocytes of the bronchial lymph node, the tonsil as well as in the lung tissue (alveolar and bronchiolar macrophages and epithelium of bronchiolar glands) of a seal stranded in France (December 18th,1999).

On the 12 seals positive by RT-PCR, fragments of the expected size of about 78 base-pair were generated from various frozen and formalin-fixed, paraffin-embedded tissues. The fragments were similar to the positive control lymph node. RT-PCR was positive for ß-actin but negative for negative control. In two cases, specific fragments were amplified from tissues of seals considered as being bycaught.

Among seals with evidence of morbillivirus infection, *Escherichia coli* was identified from blood, lung and bone marrow (3 cases), *Aeromonas hydrophila* and a haemolytic *Streptococcus* sp. were identified from the blood and brain (2 cases). These findings were consistent with a bacteremia or septicaemia.

Ref.	Body condition	Stranding date	Weight (kg)	Body length (cm)	Main necropsy findings	Main microscopy findings	Diagnostic methods	
							IHC	PCR
98/1318	2	30/07/98	8	92	Emaciation, enteritis, pneumonia	Interstitial pneumonia, lymphoid depletion	+	+
98/1321	4	12/08/98	8,5	97	Emaciation	-	NE	+
98/1354	2	26/08/98	11,5	100	Emaciation	Interstitial pneumonia, lymphoid depletion	+	+
98/1366	2	09/08/98	12,5	93,5	Emaciation, pneumonia	Acute to subacute necrotising pneumonia, lymphoid depletion, acute to subacute meningo encephalitis	+	+
98/1370	2	09/08/98	12,6	100	Emaciation, enteritis, pneumonia	Interstitial pneumonia, lymphoid depletion	+	+
	2	14/08/98	-	-	Emaciation	lymphoid depletion	-	+
98/251	4	14/08/98	11,5	-	Emaciation	-	NE	+
99/681	2	31/01/99	43	138	Lung congestion and emphysema, slight enteritis *	Lung congestion		+
99/1405	2	26/07/99	7,4	82	Emaciation, lung congestion and emphysema	Acute to subacute meningo encephalitis, lung congestion	+	+
00/132	2	16/10/99	13,5	106	Emaciation, enteritis, acute pneumonia	Lung congestion		+
00/508	2	29/12/ 99	13	95	Emaciation, acute pneumonia	Acute necrotising pneumonia	+	+
00/942	2	12/03/00	25	121	Pneumonia *	Slight acute to subacute pneumonia		+

Table XVI: Results of post-mortem investigations on the 12 seals positive for morbillivirus infection by immunohistochemistry and/or RT-PCR.

- : animals with evidence of net entrapment, the cause of death was diagnosed as bycatch

Large cetaceans.

<u>Gross findings.</u>

Between 1997 and 2001, 4 fin whales were necropsied. One was stranded on the Belgian coast (Raversijde, November 1st, 1997), one was trapped in a Dutch harbour (Vlissingen, January 13th, 2001) and two were found ashore in the northern part of France (Wimereux, October 10th, 1998; Dunkerque, November 21st, 1999). All were immature females, aged about 1 year old.

In the abdominal cavity of the first whale, a chronic thrombus (75 cm in length and 20 cm in diameter) partly obstructing the portal vein was observed. In 2 cases, necropsy was not performed due to advanced decomposition or lack of heavy equipment to handle carcasses. The 3 stranded whales were emaciated with a reduced blubber layer while the animal trapped in the harbour was in a good nutritional status.

<u>Histopathology.</u>

The mesenteric and mammary lymph nodes of one whale contained clusters of multinucleated syncytial cells with occasional large eosinophilic intranuclear inclusion bodies. The lymph nodes were severely depleted. Another whale had an focal acute to subacute inflammatory reaction in the subcutaneous tissues, characterised by neutrophils, lymphocytes, many macrophages and small numbers of syncytial cells.

<u>Morbillivirus detection.</u>

Two whales had evidence of morbillivirus infections characterized by specific intracytoplasmic and/or intranuclear labelling with anti-CDV and/or anti-PDV monoclonal antibodies in multinucleated syncytial cells and in mononuclear cells. In the subcutaneous lesions of whale 2, granular intracytoplasmic staining was observed in mononuclear cells with anti-PDV monoclonal antibody. Small numbers of intranuclear inclusion bodies, not detected by HE staining, were heavily labelled. Other evidences of morbillivirus infection were based on serology (CDV neutralizing antibody with titres of 64 and 32), electron microscopy (intranuclear aggregates of viral nucleocapsids), cell culture (viral particles (125 nm diameter), typical of paramyxovirus identified in 1 whale), and RT-PCR.

Discussion.

Small cetaceans.

Severe emaciation, multisystemic parasitism and bronchopneumonia were the most frequent findings in porpoises stranded along the Belgian and Northern French coasts. Fatal conditions observed in this study were generally similar to those described in porpoises from other regions of the North Sea (Baker and Martin, 1992; Kirkwood et al. 1997; Siebert et al. 2001). However, unlike some of these investigations, entanglement in fishing nets was a relatively unfrequent cause of death in our series. As reported by Siebert et al. (2001), determination of the cause of death is very difficult in free-ranging wild animals, and particularly cetaceans. They frequently strand when severely ill and many lesions, some considered lethal, can be observed simultaneously (Baker and Martin, 1992).

Emaciation.

The blubber thickness of emaciated porpoises in this study was significantly thinner than in animals killed in a commercial fishing operation (Read, 1990) or collected for necropsy (Lockyer, 1995). On the German North Sea coast, most stranded or bycaught porpoises were in moderate or poor nutritional state whereas animals from the Baltic Sea were in a good nutritional condition (Siebert et al. 1999, 2001). Nevertheless, stranded porpoises were in poorer body condition than bycaught animals (Benke et al. 1998). Siebert et al. (1999) considered that a poor nutritional status could be related to a reduction of food intake as a result of parasitic infection. Emaciation is not usually considered as a fatal condition for immature or adult porpoises from British waters (Baker and Martin, 1992; Kirkwood et al. 1997). Starvation was described as a cause of death only in suckling animals separated from their mothers (Baker and Martin, 1992; Kirkwood et al. 1997). In addition, these authors considered that starvation could be related to the wear and/or loss of teeth in adults. In our sample, one emaciated adult had severely worn teeth while 3 neonates were severely emaciated without evidence of milk or recent feeding in the gastro-intestinal tract. The latter finding indicates that separation from the mother should be considered as the cause of death. For other emaciated porpoises, bad nutritional status, compounded by other conditions, such as parasitosis and bronchopneumonia, should be considered fatal. In addition, a reduction of food intake can also lead to emaciation, and it is noteworthy that 70 % of the investigated porpoises had an empty gastro-intestinal tract indicating no recent food intake. It is usual that stranded cetaceans have an empty alimentary tract (Kennedy et al. 1991). It is possible that starvation was a consequence of ulceration of the upper gastro-intestinal tract. It could also have resulted from a decreased abundance of prey in the Southern North Sea which includes herring, sprat and sandeel (Addink and Smeenk, 1999). However, these fish species are still considered to be common in the area concerned.

Bronchopneumonia.

Bronchopneumonia was frequently associated with parasitosis or/and emaciation. Emaciation and parasitosis are chronic processes which could predispose to progressive debilitation leading ultimately to death from acute bronchopneumonia. Lung parasitism has frequently been reported to predispose to secondary bacterial infection resulting in bronchopneumonia. After entanglement in fishing nets, this combination has been identified as the most frequent cause of porpoise deaths in British (Baker and Martin, 1992; Jepson et al., 2000), Dutch (García Hartmann, 1997) and German waters (Benke et al. 1998; Siebert et al. 1999, 2001). In our analysis, it seems that emaciation and severe parasitosis predispose to progressive debilitation, leading to pneumonia. Acute bronchopneumonia was considered to be the cause of death of 49 % of porpoises which is similar to the 46 % reported for German animals but higher than the 10 to 12 % reported for porpoises in British waters (Baker and Martin, 1992; Kirkwood et al. 1997).

Parasitic infestations.

Heavy loads of parasites are often reported in porpoises without apparent clinical disease (Read, 1999). Nevertheless, in our study, parasitism was severe, multisystemic and associated with macroscopical and microscopical lesions. As described by Jepson et al. (2000), the airways are commonly infected by more than one parasite species and it was not possible to determine which is the most pathogenic. Nevertheless, in our investigation, lesions of bronchopneumonia were more severe around parasite larvae than around adults. Howard (1983) reported an acute reaction around larvae of *Stenurus* sp. and minimal inflammation associated with adults. Prevalence of lungworm infestation, 56 % in our investigations, is usually high in the population of porpoises of the North Sea, reaching 60 % for animals stranded on the Dutch (van Nie, 1989) and German coasts (Siebert et al. 2001) and up to 69 % for animals from British waters (Jepson et al. 2000). As suggested by Siebert et al. (2001), heavy parasitic infestation in the airways and pulmonary blood vessels may also reduce the ability to dive and hunt.

A. simplex infestation of the first gastric compartment has been frequently associated with ulceration of stranded porpoises (Baker and Martin, 1992; Brattey and Stenson, 1995; Kirkwood et al. 1997; Siebert et al. 2001). These ulcers usually have little pathological significance but could predispose for severe gastric haemorrhage leading to death (Kirkwood et al. 1997) or serve as a portal of entry for pathogens (Wünschmann et al. 1999). The prevalence of *A. simplex* in porpoises, 39 % in our study, is highly variable, with 43 % of animals from British waters (Baker and Martin, 1992), 21 % from the German coast and 15 % for Dutch animals (van Nie, 1989).

The prevalence of infection of the middle ear by *S. minor* in our investigation is similar to that reported for stranded (van Nie, 1989; Baker and Martin, 1992) and bycaught porpoises. Although no pathological change was associated with these nematodes even in severe cases (Stroud and Roffe, 1979), they could interfere with navigation (Howard et al. 1983).

Bycatch and physical trauma.

Entanglement in fishing nets seems to be a less important cause of death of porpoises in our investigations than in studies from other countries bordering the North Sea (Baker and Martin, 1992; Kuiken et al. 1994; Kirkwood et al. 1997; Benke et al. 1998; Siebert et al. 2001). In the 1993-1994 period, 7000 porpoises were estimated to have died in Danish gill nets (Vinther, 1996) and an annual mean of 6785 is reported between 1994 and 1998 (Vinther, 1999). The average of entanglement in fishing gears was 34% on the British coasts from 1990 to 1996 (Jepson et al. 2000), 50 % on the German coasts from

1991 to 1993 (Benke et al. 1998), between 10 to 20 % for the Dutch coast between 1990 and 1994 (Addink and Smeenk, 1999), and 24 % for the French part of the Channel, between 1970 and 1994 (A. Collet, unpublished data). In our study, 20 % of porpoises were considered as being trapped in fishing nets, such a level being very similar to that reported in the Netherlands. This difference between this and studies in other countries bordering the North Sea, where cetacean bycatch is considered as the largest threat for the species, could be to different fishery practices. Indeed, the use of gillnets is relatively unfrequent in Belgium and the Netherlands where the fishing fleet mainly comprises beamtrawlers (Reijnders et al. 1996). It is clear however that Dutch and Belgian waters, beyond the territorial limits, are intensively used by gill net fishermen from other European nations, especially France, Denmark and the United Kingdom. The prevalence of bycatch, if only based on the number of porpoises reported by fishermen, would be largely underestimated in the Southern North Sea. As it is asserted by Siebert et al. (2001), bycatches probably have a significant impact on population dynamics of the North Sea porpoises.

A high prevalence of infectious diseases could be related to high concentrations of organochlorines (Jepson et al. 1999) or mercury (Siebert et al. 1999) frequently reported in porpoise tissues from other parts of the North Sea. Nevertheless, such an association was not always confirmed (Kuiken et al. 1994).

A surprising observation in our investigations was a recent rise in strandings of porpoises, more particularly in 1999 and 2001 (Haelters et al. 2000; database MUMM). Our investigation failed to identify a viral infection as the cause of the mortality. An increased prevalence of entanglement in fishing nets can also be ruled out because most animals necropsied in 1999 had severe diseases with no evidence of trapping. A third hypothesis of an increased number of porpoises in the Southern North Sea, is supported by an increased number of live animals observed at sea and a recent rise in strandings in the Channel, an area where porpoises were rare previously (Van Canneyt, pers. comm.). The idea of a distribution shift of the population in the Southern North Sea should be favoured rather than a recent increase in a local subpopulation, since the ratio of juveniles to adults in our series, has not been modified. As reported by Addink and Smeenk (1999), changes in the abundance of odontocetes in certain regions can be related to the abundance of prey.

Pinnipeds.

Emaciation.

Emaciation is a frequent observation amongst seals stranded along the Belgian and Northern French coastline (Jauniaux et al. 2001) as well as on the English coast, reported as starvation (Baker, 1989), or malnutrition in live stranded animals (Barnett et al. 2000). In addition, emaciation, as observed in the present analysis, is more frequently observed on pups (Gerber et al. 1993; Baker et al. 1998). Finally, weight loss of seal pups after weaning is a normal situation and it can last for several weeks (Bonner, 1989). Emaciation may be the direct cause of death or may cause death by hypothermia by the loss of insulating subcutaneous fat (Baker, 1989; Baker et al. 1998). In our investigations, nutritional status was also related to the cause of death, animals dying of infectious diseases being severely emaciated. Morbillivirus infected dogs (Dungworth, 1993), cetaceans (Duignan et al. 1992; Kennedy et al. 1992; Jauniaux et al. 2000) and seals are frequently reported as being emaciated (Munro et al. 1992; Forsyth et al. 1998; Jauniaux et al. 2001). Emaciation in morbillivirus infected seals could be evidence of chronic disease (Baker, 1992).

Infectious lesions.

Half of the seals in our investigations showed evidences of infectious diseases. A proportion of 58 % was reported for grey seals stranded on the British coastline (Baker, 1989). Among infectious diseases on stranded seals, most frequent lesions reported were pneumonia and gastro-enteritis (Baker, 1989; Gerber et al. 1993; Baker et al. 1998).

In our analyses, bronchopneumonia, septicaemia, enteritis and encephalitis were diagnosed on many seals, most of them being infected by a morbillivirus. Severe lymphoid depletion responsible for immunosuppression, as frequently reported in morbillivirus infected pinnipeds (Krogsrud et al. 1990; Kennedy et al. 2000) can lead to such secondary diseases. Bronchopneumonia can also be secondary to lung worms (*O. circumlitus* and *Parafilaroides sp.*) infestation (Measures, 2001). But, although frequently reported in seals and more obviously in juveniles older than 3 months (Van der Kamp, 1987), few cases of parasite infestation were reported in our investigations.

During the 1998 summer, seven seals that stranded on the Belgian and French coastlines, were infected by the morbillivirus (Jauniaux et al. 2001). Since, five other seals were considered to be infected by the virus. The morbillivirus infection was diagnosed as following : specific immunolabelling, serological evidence, typical viral particles observed by TEM after cell culture and specific fragments amplified by RT-PCR. These findings suggest that a morbillivirus disease caused the seal stranding of the 1998 summer and that sporadic cases continued to occur in the southern bight of the North Sea. Among the 12 animals positive with the RT-PCR technique, only 6 seals were positive by immunohistochemistry, but many seals were too autolysed to allow immunohistochemical analyses. Indeed, the RT-PCR protocol, yielding a 78 base pair product has been developed by Krafft et al. (1995) as a diagnostic test for samples containing degraded RNA from either formalin-fixed tissues paraffin embedded tissues or from autolyzed tissues (Lipscomb et al. 1996). This corresponds to most of the samples collected from stranded marine mammals.

All morbillivirus infected seals were pups in their weaning period, corresponding with the loss of passively acquired maternal immunity, leading to a high susceptibility to PDV infection (Harder et al. 1993). As the majority of the Dutch Wadden Sea seals population, including juvenile seals which have lost their maternal antibodies, had morbillivirus-specific antibodies, it was suspected that PDV infections would continue to occur in that area (Visser et al. 1993). Finally, higher susceptibility of juveniles could be related to the immune system development (Thompson et al. 1992). Indeed, Thompson et al. (1992) reported that seropositive juvenile seals had lower antibody titres than seropositive adults.

Another reason for the emergence of seal distemper in 1998 could be the introduction in the Southern North Sea of a seal species considered as a vector. Indeed, the sudden die-off of common seals in 1988 could be related to the unusual presence of harp seals in the North Sea in 1987, seals coming from the Barents Sea (Goodhart, 1988; McGoutry, 1988; Heide-Jorgensen et al. 1992). The unusual presence of infected individuals of this species may have introduced the infection to native seal populations (Kennedy, 1998). Indeed, harbour seals in Europe were unexposed before 1988, being seropositive only after the 1988 epizootic (Thompson et al. 1992) while ringed seals and harp seals collected in Greenland were seropositive for CDV as early as 1985, without associated disease. Hooded seals along the Belgian coast, southward of their normal migratory route could also be a vector of the morbillivirus.

Non-infectious lesions.

About 50 % of the seals analyzed showed evidences of acute fatal process such as bycatch or severe trauma. In our study, only two seals were brought back by fishermen but 5 others were considered as being trapped in fishing nets on the base of gross pathology. One of those had an extensive hemothorax but also net marks. Seals are frequent victims of fishing nets but captures are not considered as a threat for these species (Northridge, 1985;1992). For grey seals, net entrapment is responsible for the death of 30 % of juveniles (Baker et al. 1998).

Another form of interaction with fisheries was the gastric perforation. Indeed, the foreign body was identified as a system used on fishing lines to separate each line from another. Similar injuries associated with foreign body ingestion such as fishhooks have been reported on seals leading in some cases to gastro-intestinal perforation, responsible for peritonitis (Gulland et al. 2001).

On the 4 seals with evidence of trauma, one was crushed on the beach by a motorbike, another one was caught in a fishing net. The origin of the trauma of the two other cases could not be determined. Trauma with extensive internal haemorrhage and fractures of skull or ribs is a frequent observation on young grey seals most of them with an unknown origin (Baker et al. 1998). As one grey seal was found in Belgian harbour, the collision with a ship seems to be the most likely cause of the trauma. Similar incidents were previously reported for seals stranded along the British coast (Baker, 1989).

Large cetaceans.

Among the 4 fin whales analyzed, 2 had evidence of morbillivirus infection that may be summarized as follows:

- Specific immunolabelling.

- Serological evidence of infection.
- Electron microscopy demonstrated large aggregates of viral material.
- Viral particles, typical of paramyxovirus, were also cultured.

To the authors' knowledge, this is the first firm report of morbillivirus disease in baleen whales. Neutralizing antibodies against dolphin morbillivirus were previously reported in serum samples of fin whales from Icelandic waters (Blixenkrone-Møller et al. 1996), and anti-CDV antibodies were detected on an adult minke whale from the Mediterranean Sea (Di Guardo et al. 1995); in neither case, however, further evidence of infection was presented.

The lymphoid depletion and the large amount of morbillivirus antigen in lymph nodes suggest that, as in other species (Lipscomb et al. 1994), the disease may be responsible for immunosuppression and secondary infections. This might explain the salmonella infection in 1 case.

Since fin whales commonly form small groups of 20 animals or less, an infectious disease is expected to spread slowly in the population. However, in feedings grounds, the groups may reach 100 or more (Gambell, 1985), thus facilitating the spread of infections, as in other cetaceans species (Duignan et al. 1995).

The area covered by the MARIN group extended from the Scheldt estuary to the Bay of the Somme. Although this region represents only a small portion of the entire North Sea coastline (200 kms), it is situated in a strategic area, where the North Sea is narrow and shallow. Such topography could result in an increased concentration of marine mammals circulating through the Channel and thereby increase the probability of drifting carcasses coming ashore.

Organochlorines and PCBs.

Organochlorines (organochlorine compounds -OC - and PCBs) are likely candidates suspected to exert long and short term negative impacts on cetacean and pinniped populations. Organochlorine compounds toxicity is in great extent due to the fact that biological systems cannot handle the carbon-chlorine bond nor split off the chlorine atoms of these compounds. They affect the nervous system by entering the nerve membrane and interfering with system functions; in vertebrates mainly the liver is affected and at a lesser extent kidneys, adrenals and skin. The acute and chronic effects include teratogenicity, carcinogenicity and mutagenicity.

The congeners that show a planar configuration are those not having chlorine atoms in ortho position. The remaining congeners adopt a nonplanar configuration being the greatest rotation effect exerted by subtitution of at least two opposing ortho-substituted chlorines in oposite rings (Boon et al. 1987, 1989; McFarland and Clarke, 1989). Planar PCBs and some of their mono- and di-ortho substituted congeners occur in very low concentrations in commercial PCB preparations only; therefore their environmental occurrence will be comparatively low in general. On the other hand, penta-, hexa- and heptachlorobiphenyls are present in high proportions in commercial mixtures; they comprise 112 of the 209 possible isomers.

Whitin the ICES 7 series (= IUPAC n : 28, 52,101,118,153,138 and 180), following congeners are considered:

- mono-ortho substituted: CB 118. This mono-ortho substitution does not eliminate, only reduces the Ah binding affinity.
- di-ortho substituted: CB 138, 153, 180. CBs 138 and 153 seem to have the greatest potential as both inducers and toxicants among di-ortho relatively planar congeners. CBs 28 and 52 do not seem to fit any dioxin-related effects.

Toxicity effects.

Experiments in animals conducted *in vivo* and *in vitro*, resulted in alteration of different enzymes and metabolic systems like enzymes of glycolysis and glucogenolysis, microsomal drug metabolism, triglyceride metabolism, oxidative phosphorilation, phospholipid metabolism, triglyceride metabolism and sterol metabolism i.e. Mixed Function Oxidase (MFO) enzyme systems. *In vitro* and *in vivo* studies have demonstrated that PCBs and organochlorine pesticides inhibit the ATPase activity, the membrane-bound enzymes which contribute to ion transport across membranes and therefore may assist as well in osmoregulation and excretion. ATPases inhibition has been observed in rabbit and rat brain, and in rat liver and kidney (Harding and Addison, 1986).

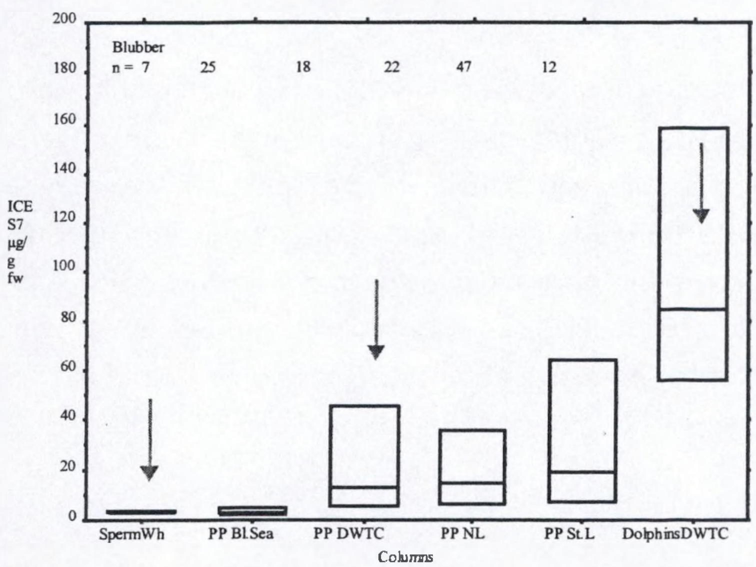
Laboratory experiments have demonstrated that PCBs cross the placenta, but no teratogenic effects on reproductive structures either in males or females have been observed, other malformations like cleft palate, cleft lip, brachydactyly, syndactyly and histological changes in the central nervous system have been induced by prenatal exposure. Functional and behavioral sterility has been induced by hormonal alterations after PCBs administration to neonatal animals.

Different experiments on females from different mammal species with chronic low levels exposure showed that PCBs induce estrogen activity, modify uterus weight, induce precocious puberty features, increase the oestrus cycle length, reduce the number of follicles, increase in the menses and menstrual bleeding and decrease overall reproductive performance i.e. abortion, embryo resorption, stillbirths; reduced offspring survival to weaning and as well as maternal death.

Some field studies on the PCBs contamination effects in wildlife have been carried out, mainly in marine mammal populations. In Californian sea lions premature births were associated with high organochlorine pesticide and PCBs levels of 1000 ppm wet weight in blubber (Harding and Addison, 1986). In ringed seals from the Baltic, uterine occlusions and subsequent foetus resorption have been correlated with high DDT and PCB levels (Helle et al. 1976; Helle, 1980). Higher juvenile mortality and lower pup production in Dutch, Danish or North German harbour seals could be attributed to higher PCB (but not to higher DDT) levels (Reijnders, 1980, 1986).

Results.

PCB concentrations in blubber of harbour porpoises from the Southern North Sea, expressed as ICES 7, range in between 1.25 and 36 µg/g on a fresh weight basis (n=32), with CBs 153, 138 and 180 covering over 90 % of ICES 7 in all tissues and CB 153 in particular ranging in between 33 and 53 % (Figure 19). These values are of a same order as the one recorded earlier for the same zone (van Scheppingen et al. 1996) in harbour porpoises stranded on the English coasts (Kuiken et al. 1994; Jepson et al. 1999) as well as in in e.g. seals from the equally polluted Bay of St. Laurent, Canada (Bernt et al. 1999). By far higher concentrations were recorded in the set of larger dolphins (blubber 64 to 183 µg/g fw, n=23). In general, they are expected to show a longer life span and therefore to accumulate higher amounts of POPs. Exact ages were, however, not available. Our data compare to very low pristine data as recorded for harbour porpoise from the Greenland and e.g. the Black Sea areas (blubber 1-5 µg/g fw; Tanabe et al. 1997; van Scheppingen et al. 1996; Holsbeek et al. in prep.). PCB levels in 7 sperm whales trapped in the North Sea that were finally to strand on the Belgian and the Dutch beaches, were equally very low, with ICES 7 values in blubber ranging from 2.3 to 3.8 µg/g fw (Holsbeek et al. 1999) and an equally different PCB fingerprint (CB153 23-29 %). On the other end of the scale are e.g. the extreme high values recorded in the literature for Mediterranean striped dolphins affected by the 1990-92 epizootic with average values in blubber, recalculated as ICES 7, of 210 µg/g (females) and 410 (males) on a fresh weight basis (Aguilar and Borrell, 1994; Borrell et al. 1996).



1 sperm whale S North Sea (DWTC)
2 harbour porpoise Black Sea (Holsbeek et al in prep)
3 harbour porpoise S North Sea (DWTC)
4 harbour porpoise S North Sea (van Scheppingen, 1996)
5 harbour seal St. Laurent (Benke et al., 1998)
6 dolphins stranded S North Sea (DWTC)

Figure 19: PCBs in marine mammal blubber (μg/g fw): median and min-max values. 1, 3 and 6 North Sea samples: Sperm whale, harbour porpoise (PP) and dolphins; 2, 4 and 5 reference data from the Black Sea (Holsbeek et al. in prep.), harbour porpoise the Netherlands (van Scheppingen, 1996) and harbour porpoise Bay of St. Laurent (Benke et al. 1998).

PCB fingerprints.

PCBs were investigated using the ICES 7 as a reference. Apart from a quantitative discussion, the distribution pattern of the individual congeners can help us to determine not only differences in trophic feeding, but also geographical differences, e.g. whether or not cetaceans belong to the Southern North Sea population, or were merely migrants, eventually bound to strand on the Southern North Sea coast.

A qualitative fingerprint analysis (Figures 20 to 22) shows that, whereas the relative fingerprint of guillemots and harbour porpoises are relatively close, with CBS 118, 153, 138 and 180 making up to 95 % of all congeners. In all cases, patterns are by far not identical, but relatively close to a Aroclor 1260 pattern. Even when originating from an initial 1254 or 1260, different metabolisation capacities for different congeners are bound to yield a pattern that does no longer fits the original pattern. Therefore, it does not make any sense to express data as 'total PCB'. The congener pattern for North Atlantic sperm whale clearly differs from all patterns in all tissues of Southern North Sea marine mammals (and seabirds) with a higher proportion of CB101 in all tissues.

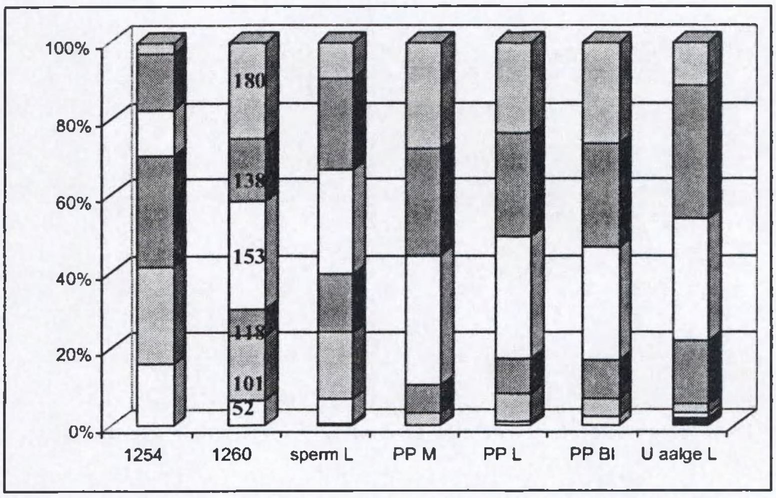


Figure 20: PCBs relative fingerprint (ICES 7) in harbour porpoise (PP, n = 25) from the Southern North Sea, common guillemot median liver (n = 202) and sperm whale (n = 7; Holsbeek et al. (1999). ICES 7: from top to bottom CB 180, 138, 153, 118, 101, 52 and 28.

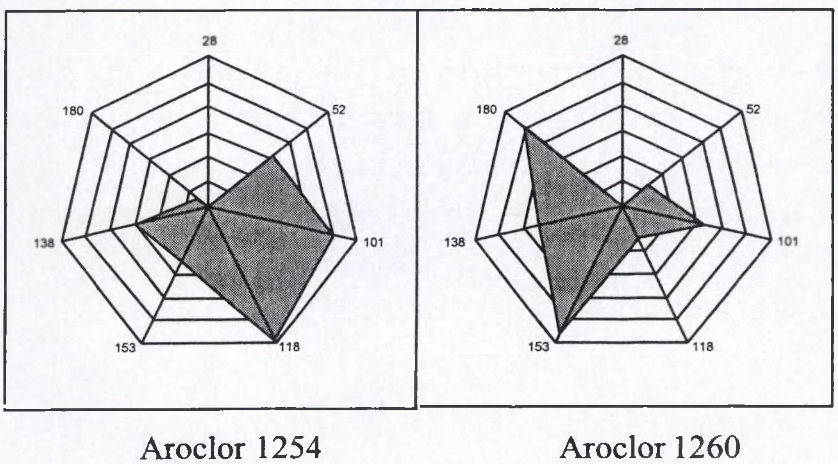


Figure 21: PCBs relative fingerprint (ICES 7) for reference materials Aroclor 1254 and 1260.

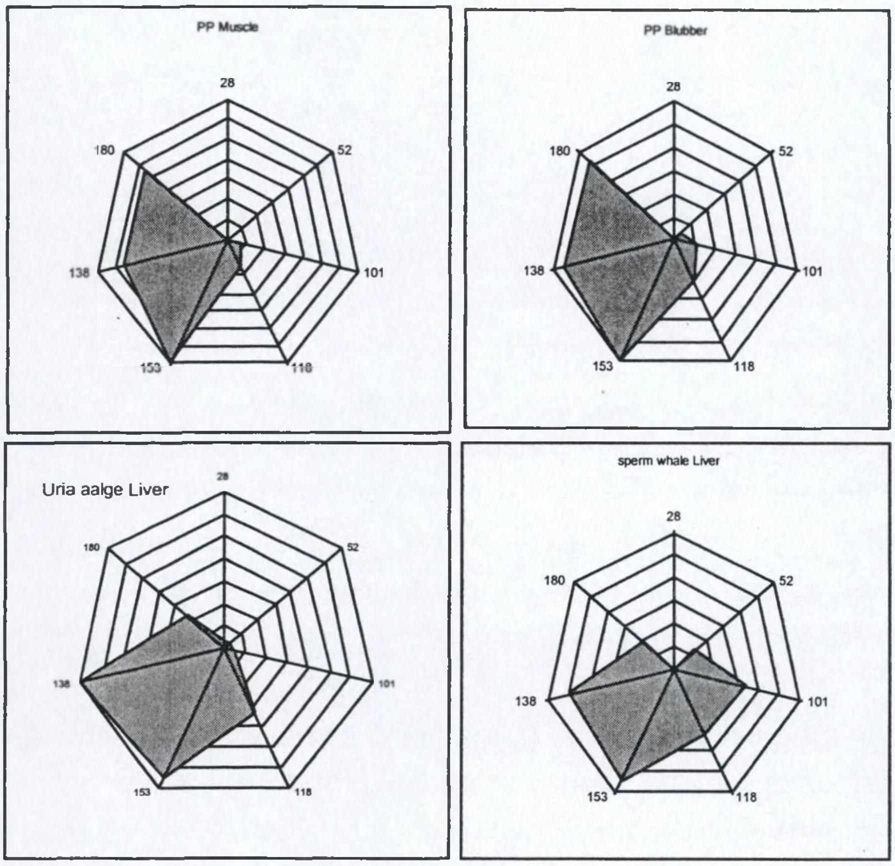


Figure 22: PCBs relative fingerprint (ICES 7) for harbour porpoise (PP, n = 25) blubber and muscle, median value, as compared to common guillemot and sperm whale.

Mercury (Hg) and methylmercury (MeHg).

Results and discussion.

Regional variations of trace metal concentrations: Hg (adapted from Holsbeek et al. in prep.)

The harbour porpoise is the most obvious species to discuss regional differences, taking into account the abundance of data. Figure 23 reviews the available data for liver total mercury ($\sum$ Hg) concentrations for harbour porpoise from the NW Atlantic and NE Atlantic regions as well as from the Black Sea. Few data sets, however, include reliable data on individual dental age and/or methylmercury (MeHg) determinations. Discussions on regional and species differences will be dealt with on a fresh weight basis in order to allow comparison with literature data.

Without taking into account the age distribution of the populations involved, harbour porpoise samples from west Greenland, the Northeast Atlantic, the Northern North Sea (Scotland, Norway, Irish Atlantic coast) and the Black Sea are clearly at the lower end of the concentration range, with samples from the Bay of Fundy, Canada and the Southern North Sea (mean values particularly high) and the Irish Sea (low average, high maximum readings) at the other end.

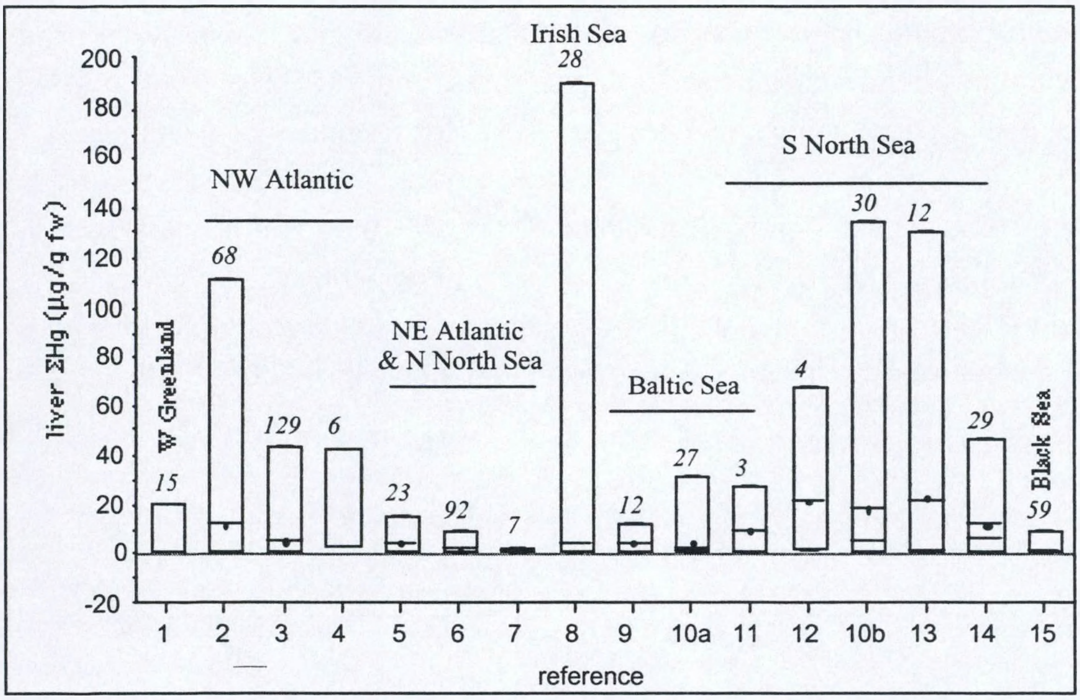


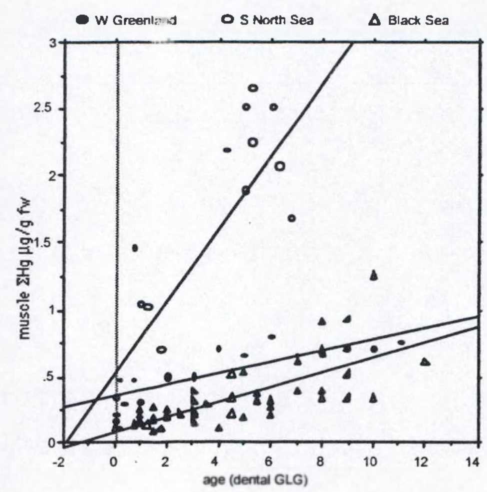
Figure 23: $\sum$Hg liver concentrations in harbour porpoise from different regions. Median (bar simple) values when available, average (bar with dot), minimum and maximum values (Holsbeek et al. in prep.). 10b = Southern North Sea.

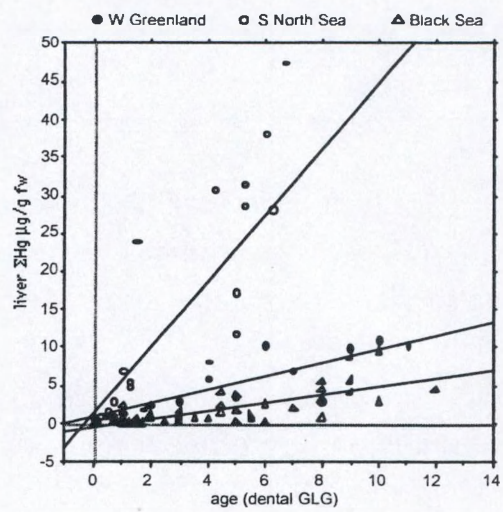
Standardisation for age is essential when comparing Hg levels in marine mammals. The age distribution of the populations involved, when available, varies considerably between samplings.

Figures 24 (muscle) and 25 (liver) relate $\sum$Hg accumulation in harbour porpoise with age, using simple regression on a numerical (a) and a semi-logarithmic (b) scale for the population from west Greenland (1, median values only; after Paludan-Müller et al. (1993), the Southern North Sea and the Black Sea. Liver concentrations do not follow a strictly linear increase: a slightly slower concentration increase in young animals probably results from a growth dilution effect.

Although there is strong reason to assume that, at least for muscle, the West Greenland data seem to go towards a plateau after age 5, as a general approach, plots are equally described as linear regressions. As proven by own striped dolphin and common dolphin data and striped dolphin data by Itano et al. (1984a), muscle and kidney MeHg and $\sum$Hg concentrations tend to stabilise at higher ages and high Hg levels. When discussing regional aspects, it might therefore be wise to focus on liver concentrations.

Despite the previous conclusion by Paludan-Müller et al. (1993) that no difference in harbour porpoise tissue $\sum$Hg concentrations exist when comparing the Southern North Sea Joiris et al. (1991) to west Greenland, a difference of approximately a factor 4 to 6 was established between both populations (regression slope liver 3.8 vs 0.9; muscle 0.26 vs 0.04 ; Table XVII).





Figures 24 and 25: Total Hg (μg/g fw) as a function of age in harbour porpoise from W. Greenland Paludan-Müller et al. (1993), the Southern North Sea and the Northern Black Sea (Joiris et al. 2001).

species	nr	origin		*n*	a	b	r^2	
HP	1	West Greenland	bc	*15*	0.89	0.82	0.69 *	Paludan-Müller *et al.*, 1993
HP	2	Bay of Fundy	bc	*68*	2.56	0.99	0.65 *	Gaski Et al. 1979
HP	6	Norway	bc	*92*	0.47	1.21	0.58 *	Teigen *et al.*, 1993
HP	10a	Baltic Sea	bc+s	*24*	2.98	-0.18	0.58	Siebert *et al.*, 1999
HP	10 b	Southern North Sea	bc+s	*22*	3.37	4.39	0.41	Siebert *et al.*, 1999
HP	14	Southern Nort Sea	s	*24*	4.35	0.73	0.63	this study
HP	15	Black Sea	bc	*54*	0.55	-0.27	0.59	Joiris et al.,2001
SD		NE Atlantic	bc	*18*	8.61	-10.7	0.88	Holsbeek et al ., IP
SD		NW Pacific	bc	*50*	9.98	0.34	0.88	Itano *et al.*, 1984
SPD		NE Pacific	bc	*40*	7.52	-11.5	0.48	Andre *et al.*, 1991
CD		NE Atlantic	bc	*10*	4.76	-0.46	0.88	Holsbeek et al ., IP
CD		French Atl. coasts	s	10	5.93	-22	0.61	Holsbeek *et al.*, 1998

Table XVII. Linear regression analysis parameters in liver of porpoises and dolphins. Species: HP harbour porpoise 1 Southern North Sea, HP Black Sea, SD striped dolphin pooled sample NE Atlantic and partial after Itano and Kawai, (1981), SPD Pantropical spotted dolphin (André et al. 1990) and CD common dolphin pooled sample NE Atlantic and French Atlantic coast (Holsbeek et al. 1998). Bc: bycatch, S:stranding.

Figure 26 shows the combined regression slopes for the 7 data sets of harbour porpoises with available age. We may assume that no major differences in uptake and excretion mechanisms exist between the different populations. Therefore, we may conclude that regional differences of Hg exposure are considerable, up to a factor 7, separating the highest (Southern North Sea) and lowest (Black Sea) exposed populations. It would be interesting to compare these results with data from other populations with known high exposure, e.g. from the Irish Sea (Law et al. 1992, ages not available), more in particular animals from the Bay of Liverpool and Cardigan Bay.

As to the reasons of high or low exposure, we may assume to some extend higher or lower background levels to be in part responsible. Up to how far Hg levels in the upper layer of the Black Sea water mass reflects e.g. the overall contamination of the system, remains unclear. A profound discussion on the topic can, however, only be made when detailed information on prey choice data is available (as for differences North Sea - Baltic Sea: Siebert et al. 1999). Since this is not the case, we will make no further attempt to do so.

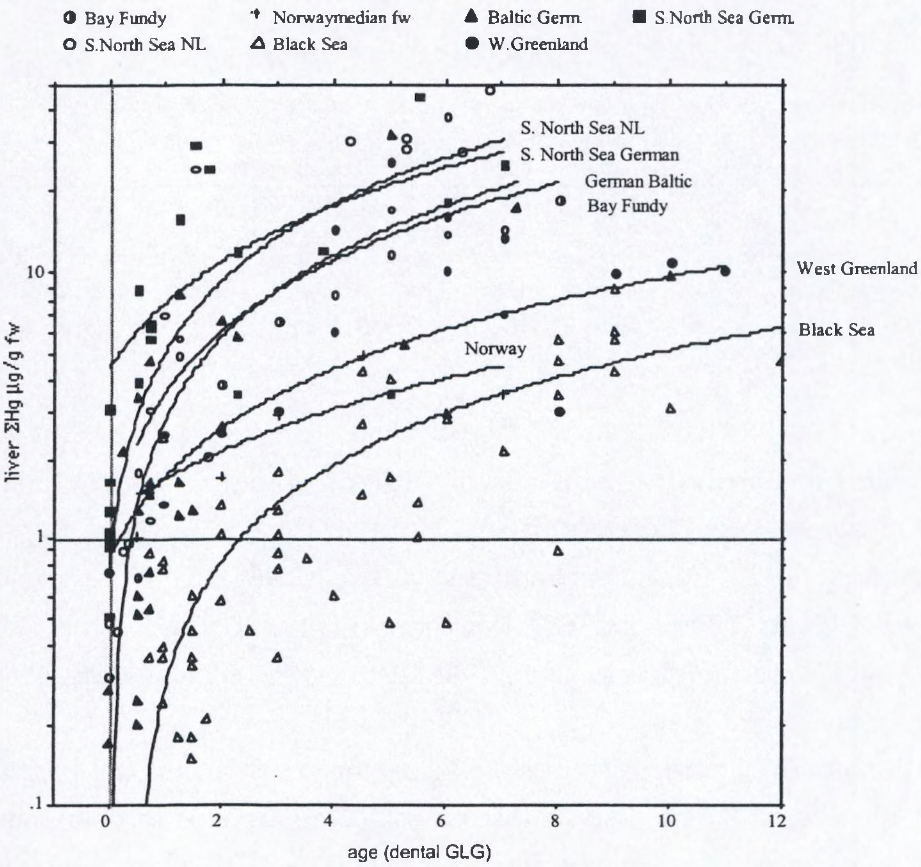


Figure 26: Total Hg (µg/g fw) as a function of age in liver of harbour porpoise from 7 locations in the Northern Atlantic and adjacent regions. Semi-logarithmic scale; linear regression analysis.

Interspecies variations of trace metal concentrations: Hg (adapted from Holsbeek et al. in prep.).

Figure 27 compares liver $\sum$Hg data for striped dolphin (SD) and common dolphin (CD) bycaught in NE Atlantic waters, common dolphin stranded on the French Atlantic coasts (Holsbeek et al. 1998) to literature data on striped dolphin caught off the Japanese coast (Itano and Kawai, 1981; Itano et al. 1984a, 1984b) and Pantropical spotted dolphin (SPD) caught off the Peruvian coast (André et al. 1990). Data sets do not fit the minimal requirements to perform a Baily significance test of regression.

There are no major differences in liver Hg accumulation patterns. Hg accumulation in striped dolphin liver seems to follow the same pattern for the NE Atlantic and NW Pacific series (slope 8.6 and 9.98, Table XVI), concentrations for spotted dolphin from the Peruvian coast are characterised by a slightly lower slope (7.5). Both batches of common dolphin stranded on the French Atlantic coast in between 1977 and 1990 (Holsbeek et al. 1998) and bycaught in the Northeast Atlantic during 1993 show similar accumulation curves (slopes 5.93 and 4.76) that fall below the curves for striped and spotted dolphin.

Northeast Atlantic common dolphin and striped dolphin, both bycaught during the same sampling, show different accumulation patterns. Whether or not this results from differences in size or prey choice, MeHg biotransformation capacity or even relative organ size, remains unanswered. Both species were, however, proven to differ slightly when it comes to prey choice and trophic positioning (Das et al. 2000c).

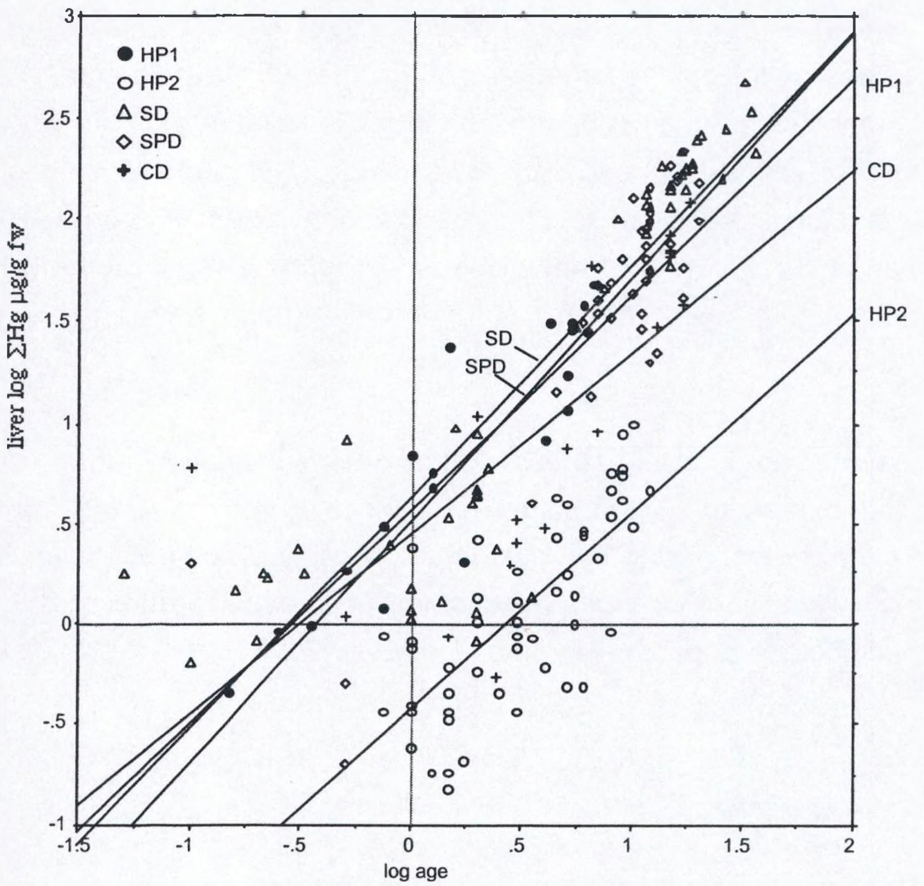


Figure 27: Total Hg (µg/g fw) as a function of age in liver of dolphins with reference to literature data. Harbour porpoise HP1 Southern North Sea, HP2 Black Sea, SD striped dolphin pooled sample NE Atlantic and partial (Itano et al. 1984a, 1984b), SPD Pantropical spotted dolphin (André et al. 1990) and CD common dolphin pooled sample NE Atlantic and French Atlantic coast (Holsbeek et al. 1998). Linear regression analysis on double logarithmathed scaling.

Figure 28 shows liver ∑ Hg concentrations as a function of age for a selected series of data, covering 4 species and 3 genera. Included are harbour porpoise series from the Southern North Sea (HP 1) and the Black Sea (HP 2), pooled data on striped dolphin (SD) from the NE Atlantic and the E. Pacific (Itano and Kawai 1981), pooled data on common dolphin (CD) from the NE Atlantic and the French Atlantic coast (Holsbeek et al. 1999) and data for pantropical spotted dolphin (SPD) caught off the Peruvian coast (André et al. 1990).

When comparing different species, one has to deal with additional problems such as the different relative age distribution of porpoises and dolphins. It seems not possible to compare in detail different species, given the differences in metabolic needs, but also in relative organ weight, both influencing liver Hg concentrations in opposite directions. One correction that can be made is for relative age, hereby partially compensating for the lower life expectancy but higher metabolic needs of smaller species. Figure 6 is a best possible attempt to correct for species relative age only. In general terms, the "normal" life expectancy for harbour porpoise (HP) is taken at 15 years, the life expectancy for the three larger dolphin species (striped dolphin SD, spotted dolphin SPD and common dolphin CD) at 40 years, resulting in a 0 to 1 relative age scale. For comparison reasons, literature data were added for minke whale MW (Hansen et al. 1990) and fin whale FW (Sanpera et al. 1993, 1995) as well as a schematized representation for long-finned pilot whale PW (Caurant et al. 1996). Due to the low number of scattered data, no regression analysis was done for baleen whales.

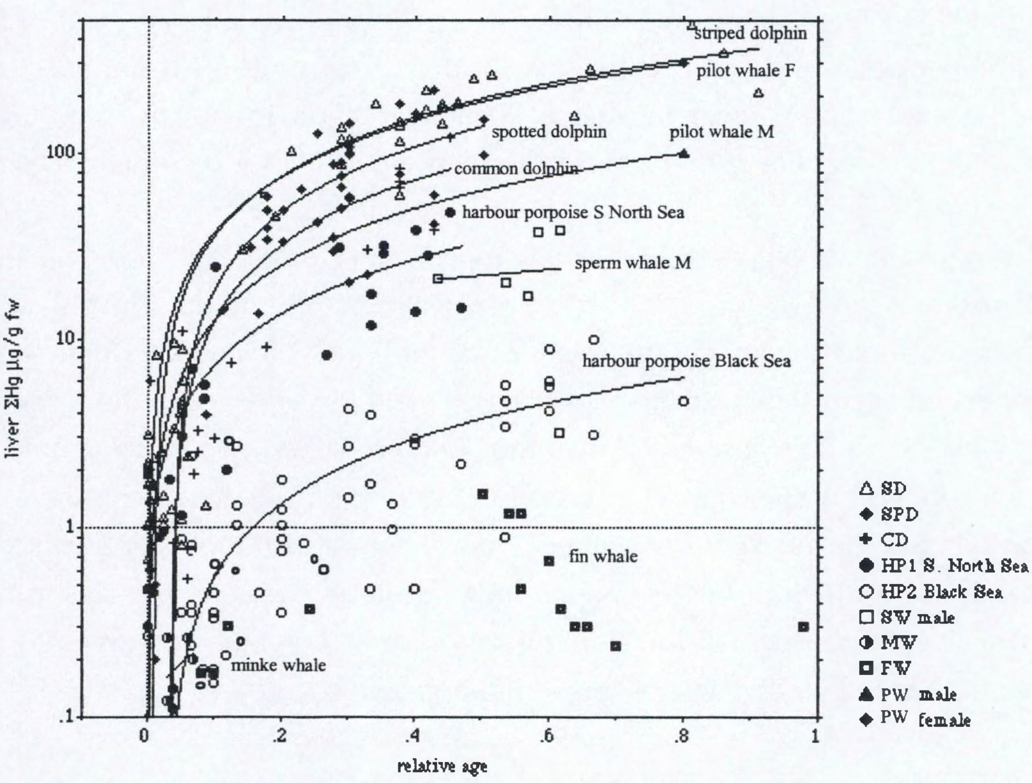


Figure 28: $\sum$Hg (μg/g fw) as a function of relative age in liver of cetaceans with reference to own and literature data. Harbour porpoise HP1 Southern North Sea, HP2 Black Sea, SD striped dolphin pooled sample NE Atlantic and partially (Itano et al. 1984a, 1984b), SPD Pantropical spotted dolphin (André et al. 1990), CD common dolphin pooled sample NE Atlantic and French Atlantic coast (Holsbeek et al. 1998), sperm whale SW (Holsbeek et al. 1999), North Atlantic baleen whales (Mysticeti: minke whale MW, this study and partially after Hansen et al. 1990); fin whale FW (Sanpera et al. 1993, 1995) and long-fin pilot whale (Caurant et al. 1994). Linear regression analysis on a semi-logarithmic scaling. M male, F female.

One may conclude that, on a relative age scale, $\sum$Hg accumulation in liver of Black Sea harbour porpoise is even lower when compared to all other series of samples. It is also remarkable that the relative concentration increase in liver of Southern North Sea harbour porpoise -which is suspected to live in a highly polluted and coastal environment- is lower than for the three, larger-sized open ocean dolphin species. There are few possible hypothesis to explain this unexpected result. Major inter-species differences in MeHg metabolization are unlikely, but not to be excluded. A difference in prey choice (smaller prey) might lead to an overall lower exposure in smaller species. Another possible explanation might be that a linear correction for relative age (age divided by normal maximal life span) does not compensate in full for the differences in metabolic demands: e.g. that a species twice as large might live twice as long (or relatively longer due to its bigger size) but has to consume more than just half the relative food intake. A final factor of influence when discussing pollutant levels on a concentration basis, is most definitely the relative size of organs, including kidney and liver: a larger relative organ size in smaller species will yield lower concentrations, even when dealing with the same exposure and liver total burden. When discussing pollutant levels on a concentration basis, with no information on liver or kidney weight, there is no way to compensate for this factor. Whether or not this has also implications on the toxicology at the level of liver or kidney (higher/lower dose of exposure) remains to be seen. The lower liver to muscle ratio in harbour porpoise as compared to dolphins is another argument to plead for this type of "organ dilution" effect.

For comparison reasons, the figure above also includes information on sperm whale (Holsbeek et al. 1999) and baleen whales from the Northeast Atlantic (minke whale: this study; partially after Hansen et al. (1990); fin whale: partially Sanpera et al. (1993)). The maximal age expectancy used to calculate relative age were 50 years for minke and fin whale, 60 years for sperm whale and 50 years for long-fin pilot whale. Detailed data for baleen whales were not available in the literature; a partial reconstruction was made on the basis of graphics and tables (Hansen et al. 1990; Sanpera et al. 1993, 1995). Minke whale ages for the small sample from this study were roughly estimated on the basis of body length (all animals < 3.5 years). The ages used for the 7 sperm whales that stranded on the Belgian and the Netherlands coasts during the winter of 1994/95 are again rough estimates. Real ages might be off by as much as 10 years or 0.16 on the relative age scaling.

As could be expected and due to their (partial) filter-feeding diet, and as shown by the few analysis on specimens stranded on the Belgian beaches, baleen whale fall well below all other Hg accumulation curves. The situation for minke whale, which is known as a mixed feeder on fish and zooplankton is less obvious. Liver concentrations seem to fall, however, in the lower half of the semi-log age accumulation curves. Differences between male and female pilot whales result from a growth dilution effect (larger males), but might as well partially result from diet differences (Caurant et al. 1994, 1996). The cephalopod specialized sperm whales, a bit unexpectedly for animals normally living very far off the coast, fall within the same order as harbour porpoise from the Southern North Sea. Again, relative organ sizes might be very important but are not included in this evaluation.

An overall conclusion might be that, despite considerable species differences, geographical variations within the harbour porpoise populations are by far more important, leading to highly significant differences in Hg accumulation patterns. Long distance-travelling species, such as sperm whales and baleen whales are less expected to show important geographical variations, taking the vast zone they cover into consideration. Smaller and coastal species, showing local migrations only, can be expected to show a more clear geographical variation.

At this point, there is no indication to suggest inter-species differences in Hg uptake and excretion mechanisms. The harbour porpoise is known as a strictly coastal species and therefore suspected to be subjected to higher doses of pollutants. This feature does, however, not show from our data. Despite the fact that smaller sized marine mammals are due to consume relatively larger amounts of food, the harbour porpoise does not show higher Hg accumulation rates. A relative high organ size might in part compensate for this. Whether or not a high prey intake in weight is compensated for by taking smaller and so possibly lower contaminated prey, remains an open question. Given the fact that both striped dolphin and common dolphin were sampled at the same time at the same place (NE Atlantic sample), differences in Hg accumulation patterns are probably due to different prey choice, as indicated by their different trophic positioning (Das et al. 2000c).

Mercury half-life in cetaceans: a re-evaluation (adapted from Holsbeek and Joiris, 2002 submitted).

Marine mammal Hg biological half-lifes (T1/2) are widely cited and accepted to range in between 500 days and 1000 days. These figures are based on a methyl^{203}Hg labeling experiment on live harp seal (Tillander et al. 1972) and actual Hg concentrations in tissues of a large series of bycaught striped dolphins (Itano and Kawai, 1981). The seal feeding experiment by Tillander et al. (1972) made use of a specially designed whole-body ^{203}Hg counting technique on a living ringed seal. Itano and Kawai, (1981) based their estimation of dolphin whole body Hg half-life on age-based accumulation slopes; tissue concentrations were recalculated into whole body burden based on relative weight of the different tissues. Gaskin et al. (1979) describes marine mammal half-life in the range of 700^{+} days without much details on how this figure is reached. Biological half-lifes in between 500 and 1000 days in a one-compartment uptake/excretion system correspond to a daily excretion of respectively 0.14 and 0.07 %. Compared to a half-life of 50 days (1.6 %/day) in humans as re-evaluated by Smith et al. (1996). This implies that within an equal time marine mammals accumulate by far larger amounts of Hg, which, as such is actually true. Indirectly, a 10 fold increased half-life might have toxicological consequences when leading to high tissue and brain levels.

The discussion on MeHg half-life in cetaceans is based on calculations on a whole body burden basis. Total Hg whole body burdens for harbour porpoise from the Southern North Sea at age 7 are estimated to average 180 mg, of which 80 mg is present as tiemannite (HgSe), basically in liver. Some of the body burden calculations as described in the literature (André et al. 1991) were proven incorrect, the importance of the blubber compartment being overestimated by at least a factor of 5. Concentrations in blubber were found to be very low, suggesting a relatively low affinity of MeHg for the lipid compartment.

Following the general characteristics of uptake/excretion models, total and methyl mercury levels in all tissues of marine mammals were described to reach a plateau with age. Own results do not confirm this finding. A concentration equilibrium as described in the literature for total mercury in liver, was proven to be an artifact. Given its nature of irreversible storage in hepatic tissue, tiemannite is due to increase throughout the entire life span of the individual. Liver $\sum$Hg concentrations reaching a plateau on an age-concentration curve are or an artifact, or result from a relative lower exposure of older animals in a previous period. Assuming Hg in other tissues to be basically MeHg and inorganic mercury (IHg^{2+}) -resulting from MeHg 'normal' biotransformation- an equilibrium of muscle and kidney concentrations ($\sum$Hg and MeHg) in time is to be expected in a normal uptake and excretion scheme. Even when tiemannite was reported in other tissues than liver, on a whole body basis, it does not contribute by much to the overall tiemannite (HgSe) burden.

A major part of the work re-evaluates the half-life of mercury in marine mammals, widely accepted to average in between 500 (seals, deduced from Tillander et al. 1972) and 1000 (dolphins, Itano and Kawai, 1981) days. All literature sources dealing with the extremely long half-life of Hg in marine mammals do, however, take no account of the large bulk of Hg that enters the system as MeHg but is then stored indefinitely as tiemannite in liver. Once biotransformed, HgSe and IHg^{2+} are, however, no longer relevant when discussing toxic effects. A distinction has therefore to be made between the MeHg parent compound and its biotransformation products. Half-lifes as described in the literature Itano and Kawai, (1981) were recalculated to be overestimated by a factor of 3 when considering MeHg only. A MeHg half-life of roughly 250 days, which corresponds to an average excretion rate of 0.28%/day can, however, not fit actual age-based accumulation curves. A concentration equilibrium is reached very fast as opposed to actual whole body concentrations which seem to stabilise after a number of years only. Even then, theoretically an equilibrium will not entirely be reached due to the continuous and cumulative formation of tiemannite, mostly in liver.

MeHg accumulation in marine mammals was therefore re-discussed on the basis of a 3-compartment theoretical model (Figures 29 to 35), describing MeHg and Hg^{2+} accumulation in all tissues and HgSe accumulation in liver only. The MeHg half-life in harbour porpoise was recalculated to average 50 days, bringing down earlier estimates by a factor 10 to 20. In this way, the MeHg half-life in cetaceans does not differ from whole body half-lifes as re-evaluated for man (two-compartments, 50 days, Smith et al. 1996). There is little doubt that, as an order of magnitude, this new half-life estimation applies to all marine mammals species, if not to all vertebrates of a similar size.

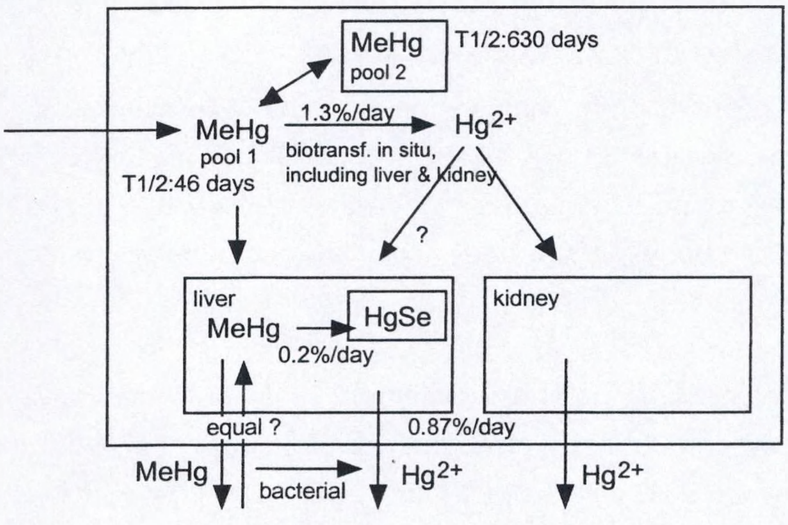
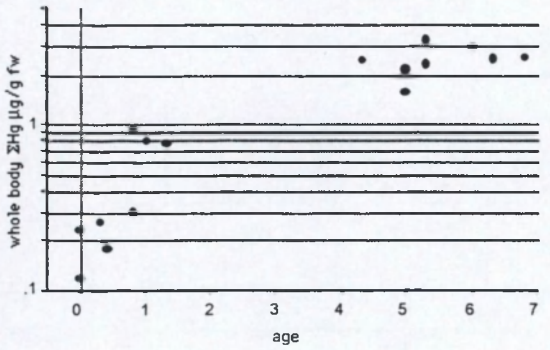


Figure 29: Uptake-excretion model II, three compartments model with half-life and flow parameters, best fitting actual data in harbour porpoise corresponding to an overall half life (50 % elimination) of 50 days.



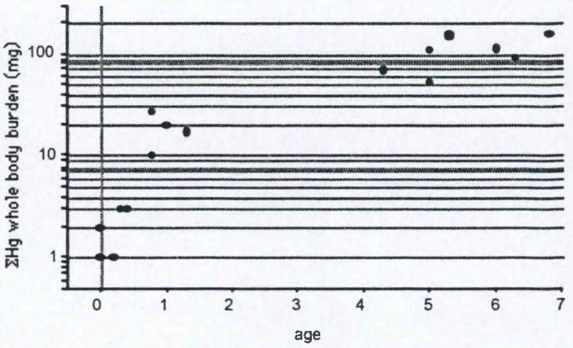
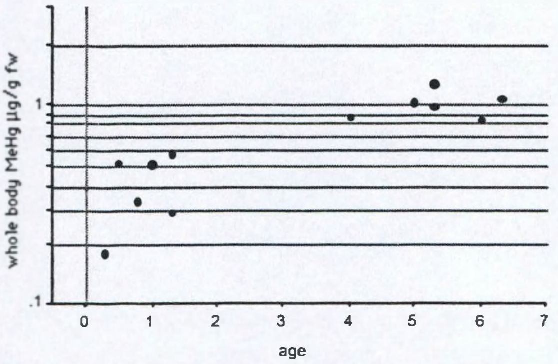


Figure 30: $\sum$Hg whole body concentration (μg/g fw) as a function of age in Southern North Sea harbour porpoise; semi-log representation.

Figure 31: $\sum$Hg whole body burden (mg) as a function of age in Southern North Sea harbour porpoise; semi-log representation.



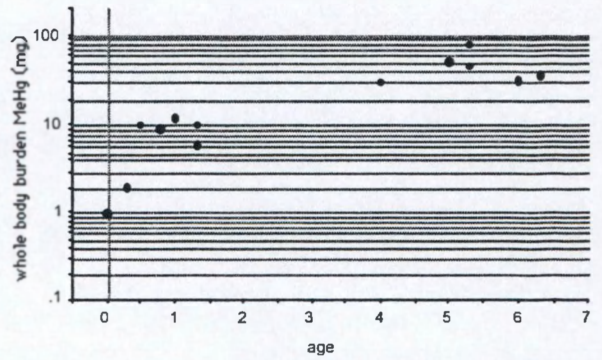
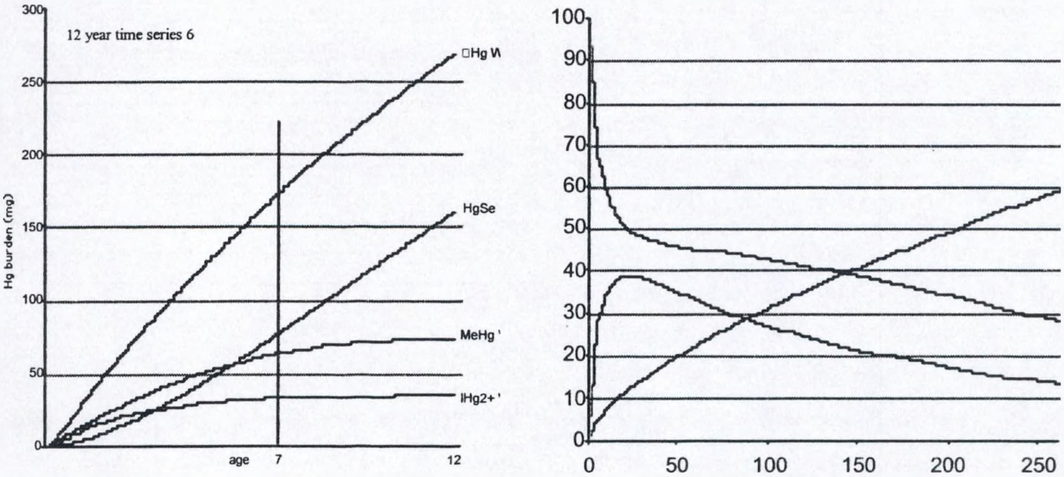


Figure 32: MeHg whole body concentration (μg/g fw) as a function of age, in Southern North Sea harbour porpoise; semi-log representation.

Figure 33: MeHg whole body burden (mg) as a function of age in Southern North Sea harbour porpoise; semi-log representation.



Figures 34 and 35: Results three compartment modelisation, fish concentration 0.1 μg/g fw; MeHg 50% half-life 50 days (pool 1 46 days, pool 2 630 days). 12 year time series, theoretical increase of whole body MeHg, whole body IHg^{2+}, liver HgSe and whole body total Hg. Evolution with age of whole body burdens (12), relative fractions (%ages) with increasing $\sum$Hg burden (13).

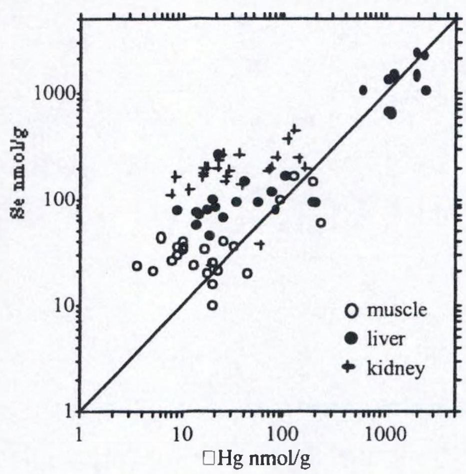
Relationship mercury-selenium *(combined discussion Ulg Oceanology and VUB Ecotox).*

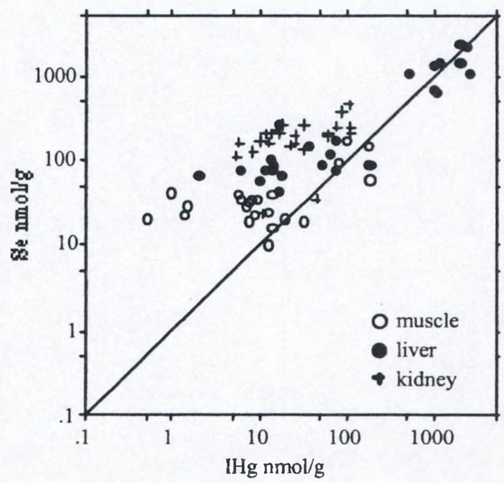
Our results confirm earlier findings on the importance of age related Hg accumulation, impact of tiemannite accumulation on speciation (decreasing percentage of MeHg with increasing loads) and inter-tissue relationships. The formation of tiemannite was, however, previously described as resulting from a two-step accumulation mechanism (Palmisano et al. 1995), a series of authors also concluding on a threshold level ($\sum$Hg 100 μg/g fw in liver) for the onset of a tiemannite-linked MeHg detoxification, basically along the idea of the reaching a Hg:Se equimolarity at higher concentration levels only. This theory proved to be incorrect, basically because being based on a circular pattern of thinking. Given the fact that hepatic levels of 100 μg/g fw can only be reached with interference of tiemannite, MeHg meanwhile remaining at low levels, the same 100 μg/g can not be used as a threshold for the onset of its own formation. The reaching of an equimolar Hg to Se ratio can be fully explained by the gradual increase of tiemannite levels in liver only. Own results and existing literature data show that molar Hg to Se hepatic ratios go towards equimolarity as a result of the slow nature of the tiemannite detoxification process, with tiemannite gradually taking the upper hand over MeHg, IHg^{2+} and a surplus of 'free' selenium with increasing loads (Figures 36 to 38).

If a threshold for the formation of tiemannite in marine mammals -and for that matter as well in carnivores and birds- would exist, as suggested by the absence of a total mercury increase with age in the tissues of baleen whales (Hansen et al. 1990; Sanpera et al. 1993) but also in seals from the unpolluted Nordkap region, Norway (Skaare et al. 1994) it would depend on tissue levels of MeHg. Own data suggest a tendency towards Hg:Se equimolarity at MeHg levels above 3 μg/g fw. Even then, inorganic concentrations in liver still include a certain amount of IHg^{2+}.

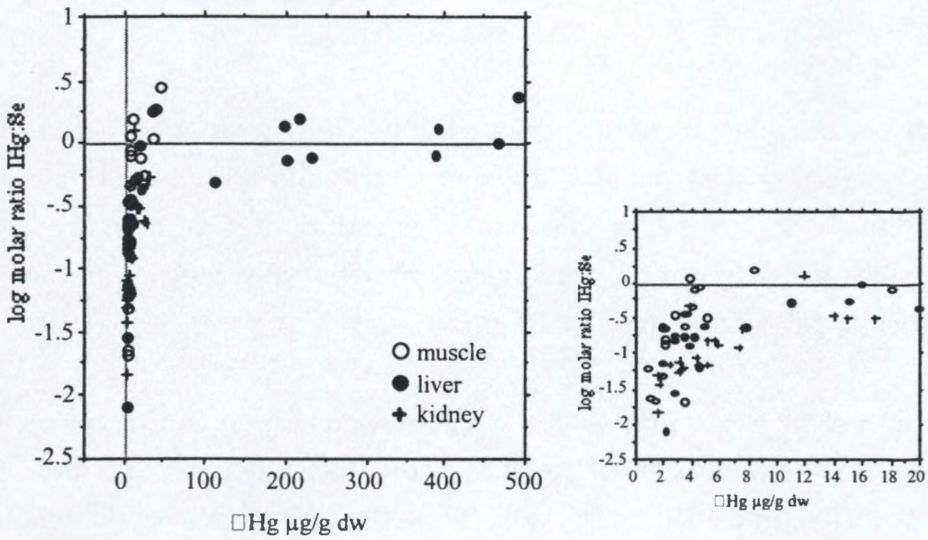
As to the formation of tiemannite outside the liver, there are arguments in favour of both opposite opinions. Small granular particles were reported in the literature in kidney, spleen, bone marrow and brain of several marine mammal species (Martoja and Berry, 1980; Augier et al. 1993a, 1993b; Nigro and Leonzio, 1993, 1996; Nigro, 1994 Rawson et al. 1993, 1995). In few cases only, the molar Hg to Se ratio does, however, reach equimolarity, with concentrations usually remaining one order of magnitude lower when compared to liver. As described in the literature, only a minor fraction of mercury in kidney seems to be bound to metallothioneins (MTs) or MT-like proteins. Relative percentages of MeHg in kidney, as well as in muscle, seem however, to follow a similar decreasing trend with increasing total mercury loads. Part of the mercury in other tissues than liver might therefore be related to tiemannite, especially in cases of high exposure. Literature sources add to this the possibility of small numbers of small-sized tiemannite particles of a second type to be redistributed from the liver to other tissues. Combined literature and own data in case of lower exposures, suggest that kidney and muscle total mercury levels reach an equilibrium after a number of years, a phenomenon that does not fit with 'in situ' and continuous tiemannite formation, except perhaps in cases of high exposure.

Based on actual estimates of MeHg exposure and tissue concentrations of $\sum$Hg and MeHg in harbour porpoise from the Southern North Sea, Hg detoxification through precipitation of tiemannite is evaluated to neutralise on average 12 % of the overall MeHg intake at the age of 7 years. Tissue concentrations were, however, proven not to increase dramatically if this type of detoxification process would not be in place. Based on the idea of a 3 fold excess in toxicity of mercury over selenium and a 5 to 15 molar concentration excess of selenium over Hg in fish, Hg seems to play the major part in the mutual detoxification.





Figures 36 and 37: Selenium (nmol/g dw) in pooled sampled of striped dolphin (n = 18) and common dolphin (n = 9). Molar relation to a. total mercury and b. inorganic Hg in muscle, liver and kidney. Line = molar 1:1 ratio.



Figures 38a and 38b: Selenium in pooled sampled of striped dolphin and common dolphin. Log molar ratio IHg to selenium as a function of increasing total body concentration (μg/g dw) in muscle, liver and kidney (a) and partial representation of the 0-20 μg $\sum$ Hg/g dw sub-sample (b).

Heavy metals.

Introduction.

During the past few decades, increasing concern about environmental pollution has led to many investigations on heavy metals and their distribution in sea, air or biological materials. Within the North Sea, heavy metals such as cadmium (Cd), mercury (Hg), or lead (Pb), remain substances for priority action under the OSPAR strategy with regards to hazardous substances (OSPAR commission 2000).

Marine mammals appear to be potentially valuable indicators of the level of heavy metals accumulated in the marine environment: according to their top position in the trophic network, their long life span and their long biological half-time of elimination of pollutants, these animals can accumulate high levels of these compounds (Das et al. in press a).

Several investigations have been carried out in an attempt to evaluate organic contaminant effects at ambient environmental levels (Reijnders, 1986; Aguilar and Borrel, 1994; De Guise et al. 1995; de Swart et al. 1994). For example, it has been demonstrated that seals fed polluted fish from Dutch Wadden Sea showed reduced pup production when compared to those fed much less polluted fish from the Northeast Atlantic (Reijnders 1986). This study was the first sign of a causal relationship between naturally occurring levels of pollutants and a physiological response in marine mammals.

However, fewer studies have tried to link marine mammal health status and metal level within the North Sea and adjacent areas (Hÿvarinen and Sipilä, 1984; Siebert et al. 1999; Bennet et al. 2001). Investigations carried out on a ringed seal population from Finland showed a clear connection between stillbirth of the pups and nickel concentrations of the air (Hÿvarinen and Sipilä, 1984). These authors have underlined the considerable nickel input in the environment from industrial activity in that particular area.

Siebert et al. (1999) examined the possible relationship between Hg tissue concentrations and disease in harbour porpoises and white-beaked dolphins from the

German waters of the North and Baltic Seas. This study showed that harbour porpoises and white-beaked dolphins from the North Sea are carrying a significant burden of mercury. The higher mercury content in organs from harbour porpoises from the North Sea indicated that mercury is a more important threat for animals of this region than for animals of the Baltic Sea. From this study it appears also that mercury burden is associated with prevalence of parasitic infection and pneumonia.

Bennet et al. (2001) have also postulated that increased exposure to toxic metals results in lowered resistance to infectious disease in harbour porpoises from the coasts of England and Wales. Mean liver concentrations of Hg, Se, Hg:Se ratio, and Zn were significantly higher in the porpoises that died of infectious diseases (parasitic, bacterial, fungal and viral pathogens such as pneumonia, compared to porpoises that died from physical trauma (most frequently entrapment in fishing gear).

Since the project PODO 1 began 5 years ago, trace metal analyses have been performed in 7 species of marine mammals collected along the Belgian coast and adjacent areas :the harbour porpoise, the harbour seal, the white-beaked dolphin, the white-sided dolphin, the grey seal, the hooded seal, and the fin whale. In the framework of this research project, factors that could be responsible for metal level variations both between different species (**interspecific** causes of variations: e.g. diet) and within a species (**intraspecific** causes of variations: diet, age, nutritional status and metallothioneins) were studied.

Material and methods.

To increase the sampling and improve the efficiency of the statistical analysis, data obtained from marine mammals (especially harbour porpoises) stranded before 1997 were integrated in the results.

Heavy metals analysis.

Zn, Cd, Fe Ni, Pb, Cr and Cu analyses: see procedure p 48-49.

In addition, Hg was analysed by flameless atomic absorption as described by Joiris et al. (1991). The Hg absolute detection limit is 10 ng corresponding to 0.13 $\mu g.g^{-1}$ fresh weight for an average of 1.5 g fresh weight.

Metallothioneins analysis.

See procedure p. 60-61.

Results and discussion.

Heavy metal concentrations depend not only on the environment contamination but also on several other biological factors such as the diet or the age (Das et al. in press a). In a first approach, trace metal concentrations have been compared between the different species of marine mammals.

Interspecific variations of trace metal concentrations (*adapted from Das et al. 2000b).*

Marine mammals display strong interspecific Cd concentrations. The highest Cd concentrations are observed in the kidney of the oceanic feeders such as sperm whales (mean: 258 $\mu g.g^{-1}$ dw, Holsbeek et al. 1999; Das et al. 2000c). Cd values are also very high for the 2 white-sided dolphins (88.0 and 88.4 $\mu g.g^{-1}$ dw) and the hooded seal (63 $\mu g.g^{-1}$ dw) while remaining relatively low in white-beaked dolphin, harbour porpoise, grey and harbour seal kidneys (Figure 39).

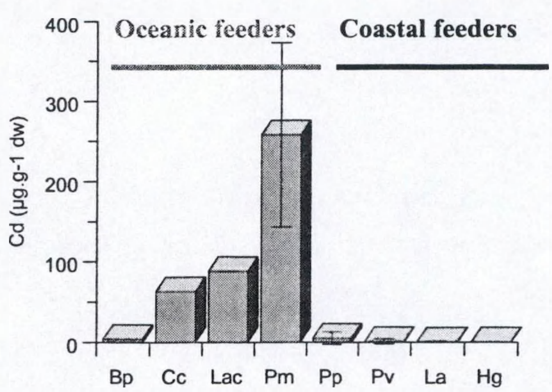


Figure 39: Respective renal Cd concentrations of North Sea marine mammals (Bp: *Balaenoptera physalus*, Lac: *Leucopterus acutus*, Pm: *Physeter macrocephalus*, Pp: *Phocoena phocoena*, Pv: *Phoca vitulina*, La: *Lagenorhynchus albirostris*, POM: Particulate organic matter, data from Middelburg and Nieuwenhuize, 1998; Cd data for sperm whales after Holsbeek et al. 1999).

The hooded seal, sperm whale and white-sided dolphin high concentrations are likely to be diet related as teuthophageous marine mammals display elevated Cd concentrations in their livers and kidneys (Bustamante et al. 1998). In agrement with this hypothesis, sperm whales, fin whales, white-sided dolphins and the hooded seal show very low $\delta^{13}C$ values compared to the four other species (Das et al. 2000b). The $\delta^{13}C$ depletion observed for sperm whales, hooded seal, fin whales and white-sided dolphins presumably reflect a greater reliance on offshore food such as oceanic cephalopods. High Cd concentrations displayed by the hooded seal may also be related to its geographic origine as this species is quite Northern (Nigel Bonner, 1989). Indeed, arctic foods chains display also high Cd levels due to specific rock layers (AMAP, 1998).

Intraspecific variations of trace metal concentrations: case of the harbour porpoise.

<u>Geographic variations of trace metal concentrations.</u>

Trace metal concentrations measured in harbour porpoises stranded on the Southern North Sea coasts were higher compared with data available from the literature. It appears that harbour porpoises from the North Sea can display high hepatic Zn (Figure 40) and Cu concentrations when compared to porpoises from the Baltic or the Black Sea.

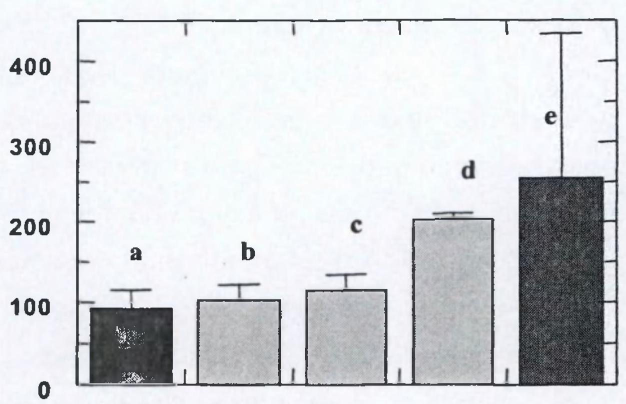


Figure 40: Mean Zn concentrations ($\mu g.g^{-1}$ dw) in the livers of harbour porpoises from the the Black Sea (**a**, unpublished data, n=40), the Baltic Sea (**b**, Szefer et al. 1994, n=4), the West Atlantic (**c**, Mackey et al. 1995, n=6), the Greenland (**d**, Paludan-Müller et al. 1993, n=44), and Southern Bight of the North Sea (**e**, this work, n=52).

Siebert et al. (1999) observed that harbour porpoises from North Sea are carrying a significant burden of mercury. The higher mercury content in organs from harbour porpoises from the North Sea indicated that mercury is a more important threat for animals of this region than for animals of the Baltic Sea. Similar conclusions were drawn for another species wintering in the Southern North Sea, the common guillemot:its high heavy metal level (Cu, Zn, Hg) in tissue could favor a debilitation process (Debacker et al. 2001).

Variation of trace metal concentration with age.

Cd and Hg accumulate strongly with the length of the animal in mostly all marine mammal tissues analysed reflecting an age accumulation. Nevertheless, a systematic age determination should provide a better understanding of this process.

Variation of trace metal concentrations with nutritional status.

Potential effects of toxic metals cannot be tested in free-living cetaceans because experimental manipulations are undesirable/illegal. One approach to this problem is to carry out systematic post-mortem investigations to establish the disease status of contaminated animals in a relatively large number of individuals from the same species. In harbour porpoises found dead along our coasts, one of the main lesions observed during the necropsy is a severe emaciation characterised by a reduction of blubber thickness and muscle atrophy (Jauniaux et al. accepted). Trace metal concentrations were compared between emaciated and non-emaciated porpoises. Hepatic Zn (Figure 41) and Se concentrations measured are significantly higher in emaciated juveniles compared to non-emaciated ones. In adults, trace metal levels are similar between emaciated and non-emaciated porpoises. Other trace metal concentrations (Cd, Cu, Ni, Cr, Pb, Hg, Fe) are similar between emaciated and non-emaciated porpoises both for adults and juveniles ($p>0.1$). Bennet et al. (2001) have also used this indirect approach to investigate the prediction that increased exposure to toxic metals results in lowered resistance to infectious disease in harbour porpoises from the coasts of England and Wales. Mean liver concentrations of Hg, Se, Hg:Se ratio, and Zn were significantly higher in those porpoises that died of infectious diseases (parasitic, bacterial, fungal and viral pathogens such as pneumonia, compared to porpoises that died from physical trauma (most frequently entrapment in fishing gear).

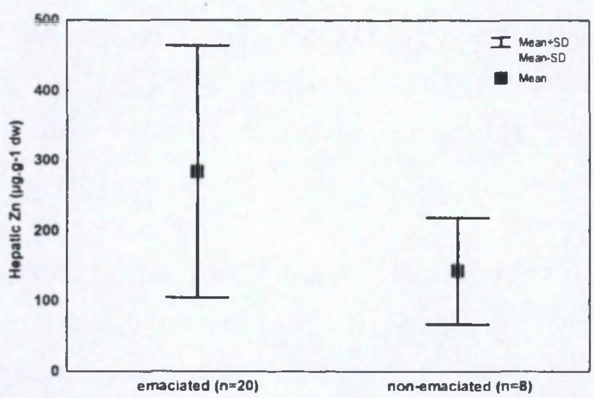


Figure 41: Hepatic Zn concentrations in juveniles harbour porpoises stranded along the Belgian coast and adjacent areas.

High Zn concentrations encountered in emaciated juvenile porpoises could be related to a redistribution of zinc from other organs such as liver or muscle. Indeed, food deprivation in rat can lead to a redistribution of the hepatic Zn due to protein catabolism and the increase of hepatic zinc concentration is related to a loss of the liver mass (Krämer et al. 1993). However during the emaciation process displayed by porpoises, no loss of liver mass has been observed (Siebert, pers. com.) indicating that the increase of hepatic Zn concentration might be related to muscle protein catabolism rather than liver.

However, Debacker et al. (2001) have shown that combined starvation and high Zn, Cu and Hg in the diet can trigger the emaciation process in Japanese quails which in turn increases Zn levels in the liver due to protein catabolism. It appears that although emaciation is linked to starvation which can influence heavy-metal level, the high levels encountered in the porpoises from the Southern North Sea could probably favour a debilitating process leading to emaciation (Debacker et al. 2001).

Role of metallothioneins in marine mammal livers and kidneys.

Few papers have focused on marine mammal metallothioneins (MTs) despite the fact these species can accumulate high levels of metals such as cadmium or mercury (Das et al. 2000a). Metallothioneins have first been characterised in the kidneys of a fresh white-sided dolphin displaying high levels of cadmium and mercury in liver and kidney (Das et al. in press b). The protein has two isoforms: MT-1 and MT-2. MT-1 binds Cu, Zn, Hg and Cd while MT-2 only binds Zn, Hg and Cd. This suggest different metabolic functions for these two isoforms: MT-1 is mainly involved in Cu homeostasis; MT-2, which was four times more abundant than MT-1, detoxifies most of the accumulated cadmium.

Further investigations on MTs function have been carried out on harbour porpoises displaying a good conservation state. Total proteins have been measured in the livers and it appears that MTs represent 1.3 % of the total protein concentrations. Despite this weak percentage, MTs appear to have a key role as in the liver they bind 50 % of the total Zn while only 19 % is present on the high molecular weight proteins. MTs are also involved in Cd detoxication as they bind 56 % of the total renal Cd. Both in livers and kidney, MTs appear to have a weak role in Hg detoxication as this metal is distributed mainly in the pellet (Table XVIII).

A significant relationship has been observed between total hepatic Zn and MT concentrations in relation with the Zn induction function in the cell.

	pellet	**soluble fraction**	**HMWP**	**MTs**	**LMWP**
Hepatic Zn n=14	86 ± 58 (30-228) **30%**	177 ± 159 (41-631) **70%**	53 ± 19 (29-104) **19%**	144 ± 165 (16-595) **50%**	1,8 ± 117 (<0.1-6,2) **1%**
Hepatic Cu n=14	14 ± 12 (6-43) **48%**	18 ± 14 (6-49) **52%**	5 ± 3 (1.3-12) **18%**	10 ± 11 (2-39) **34%**	<0,1 (<0.1-6.1) **<LD**
Hepatic Hg n=14	43 ± 51 (0.5-167) **96%**	3 ± 2 (0.4-7) **<4%**	0,13 ± 0,1 (0.01-0.3) **~0.5%**	0,08 ± 0,07 (0-0.22) **~<0.2**	0,02 ± 0,01 (0-0.05) **~<0.05%**
Renal Cd n=14	1,1 ± 1,8 (<0,1 – 5,6)	2,4 ± 2,4 (<0,1 – 5,9) **67%**	0,2 ± 0,6 (<0,1 – 0,9)	1.9 ± 2 (<0,1 - 5) **56%**	0, 2 ± 0,8 (<0,1 – 1,7)

	33%		6%		5%

Table XVIII: Hepatic Zn, Cu, Hg and renal Cd concentrations ($\mu g.g^{-1}$ dry weight) in the pellet, the soluble fraction, high molecular weight proteins (HMWP), metallothioneins (MTs) et low molecular weight proteins (LMWP).

Moreover, when Zn increases in the liver, its percentage bound to MTs increases also (from 20 to nearly 70 %), suggesting that these proteins might take in charge the Zn overload resulting from the emaciation process (Figure 42).

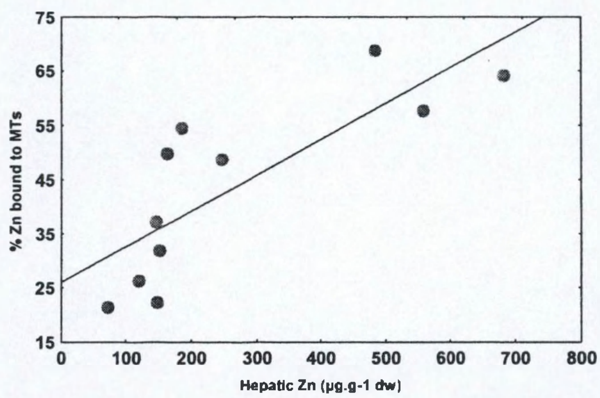


Figure 42: Relationship between total hepatic Zn concentrations and Zn bound to MTs (%).

Conclusions.

Within the North Sea, trace metal levels in marine mammals display strong intra- and interspecies variations due to geographic origin, age, diet, trophic position but also by nutritional status of the individuals. Zn, Cu and Hg concentrations were higher in harbour porpoises from the Southern North Sea compared to other areas. Some individuals were severely emaciated as shown by their muscle atrophy and reduced blubber thickness. Hepatic Zn and Se concentrations were significantly higher in emaciated juvenile porpoises than in normal animals suggesting a severe disturbance of the metal homeostasis. Metallothioneins appear to have a key role in the homeostasis of Zn and Cu and in the detoxication of Cd. On the contrary, Hg is mainly present in the insoluble fraction of the tissue in relation with tiemmanite detoxication process.

Further investigations are needed before we can reach any definitive conclusions but we cannot reject the hypothesis that trace metal exposure may influence marine mammal health and contribute to the high mortality observed these last few years.

Marine mammals : general discussion and conclusions.

Based on the data that are available at this moment, within the MARIN framework and outside, it is very difficult to identify causal relations between pathological observations and tissue levels and loads of heavy metals and POPs. A first reason for this being, perhaps, the fact that the samples that are worked on are far from at random: a lot of animals that are found on the beached died of natural causes, including internal lesions or pathologies. On top of this, it is not unusual to find a great number of pathologies, lesions, or parasites, in a population of dolphins or porpoises considered healthy. Only a minor proportion of the examined animals had been killed in fishing gear (bycaught).

The MARIN network has no access to bycaught animals from the fishery fleet, but probably only very few individuals are being caught by Belgian fishermen. Bycaught animals are useful to compare with the stranded batch.

Our data show that the marine mammals that are normal inhabitants of the North Sea are subject to a large number of environmental stress factors, not in the least heavy metals and POPs. Given their high trophic position marine mammals are subjected to daily doses which would be considered as being highly threatening if it would concern a human population. The combined effect of a relative long life span and the tendency of some liposoluble molecules to concentrate and bioaccumulate within the trophic web will eventually lead to very high concentrations of PCBs and related substances in adult animals. The fact that a major portion of POPs is passed on to the next generation through mother milk, which has a high lipid content, poses a continuous threat to the survival of the marine mammal species.

High PCBs tissue levels that were linked with immune system deficiencies and the spreading of lethal viral diseases, as reported in seals from the Wadden Islands or dolphins from the Mediterranean, were of a level of magnitude higher than the figures currently found in Southern North Sea animals. However, there is little knowledge on the thresholds that need to be reached before the onset of such a type of event. Pollutant levels prove to be highly variable within and among species and need to be corrected for a factor 'time' when considering the more liposoluble compounds.

The fact, however, that the numbers of marine mammals found on the Southern North Sea coasts, are rising, should encourage the idea that the general health status of the populations is such that no major health or reproductive implications at the population level exist. Major mechanisms of detoxification, as studied in this work, may play an important role in counterbalancing stable pollutant stress. In particular with respect to a number of heavy metals, marine mammals seem to be well equipped to deal with daily doses which seem completely unacceptable to e.g. man. Metallothioneins and metal-rich granules seem to be highly efficient mechanisms to resist to high and chronic heavy metal exposure. As to persistent organic molecules, given perhaps their more recent, anthropogenic nature, marine mammals are in general reported to be poor metabolisers. Fluxes of PCBs and other POPs towards the North Sea, and levels in marine mammal tissue should be carefully monitored, now and in the future.

As to the structure of future research, and given the difficulties to link pathology and tissue levels, it would be appropriate to monitor a number of specific biomarkers for heavy metal and POP exposure. Blood level parameters, endocrine disruption, lymphocyte activity and POP-metabolisation parameters might add important information to the knowledge that has been gained by this MARIN network and other teams.

Biomarker responses performed on either very fresh or live animals might give us important information on the reason of the live stranding of a number of on first sight healthy animals. When compared to populations that are subjected to lower levels of exposure, biomarker levels will enable to quantify pollutant stress and its relation to daily exposure and metabolisation.

GENERAL CONCLUSIONS, RECOMMENDATIONS AND PERSPECTIVES

General conclusions, recommendations and perspectives.

At the term of this 5 year research programme the MARIN group has gained further experience and progress was made at different levels. General conclusions and recommendations can be presented as follows :

Seabirds.

- An experimental approach combining both drifting experiments at sea and persistence of the beached birds' carcasses on the beach gave a first insight of how mortalities at sea relates to densities of beached birds on the coastline. It is shown that temporal patterns of beached birds usually follow those of seabirds at sea with a time-lag of at least one month. Persistence experiments carried out during three winters confirmed the picture of short residence time in the Southern North Sea. In addition, oiled birds are more persistent than other corpses. It is concluded that (i) beached numbers can be usefull and indicate interesting patterns when carefully interpreted and in an international context ; (ii) there is a need to further develop drift-, persistence- and floating experiments in order to fully understand what a bird corpse does from the moment it dies until it is being found on the beach ; (iii) special attention should be given to 'oiled' versus 'unoiled' carcasses and to what happens to corpses once they have sunk ; (iv) models should be developed to help predicting patterns of seabirds strandings (Seys et al. submitted c).

- Continuous sampling over the last 5 wintering seasons permitted to acquire a temporal trend and precise our previous observations. Additional necropsies permitted to identify major pathologies and to present a general scheme of events finally leading the bird to die. Observations of oiled beached birds permitted to complete an existing database and establish a significant decline of the oil pollution at least for some seabirds groups like the auks (common guillemot/razorbill) and the Larus-gulls (Seys et al. submitted a).

- Experimental contamination with heavy metals of different groups of common quails which were subsequently starved permitted to clarify the potential link existing between the heavy metal levels and the major lesion observed : the cachectic status.

* Extended international contacts allowed the MARIN group to largely exchange information and have access to samples of high interest such as the common guillemots necropsied after the Erika's oil spill (Brittany, France) and those which had been collected stranded on Scottish beaches.

<u>The MARIN group recommend :</u>

* To continue monitoring the health status of top predators such as seabirds at the Belgian coast.

* Apart from oil pollution which has been studied over the last years, other human related disturbances (destruction/alteration of habitats, impact of plastic debris at sea, fishing activities, ...) should be carefully assessed.

* Further research is needed, in particular to extend the scope of the research towards other pollutants such as the well known deleterious persistent organic pollutants (POPs). Preliminary analyses have already demonstrated that high levels of dioxin and dioxin-like compounds were present in the tissues of common guillemots. In addition, it would be appropriate to assess parameters such as specific biomarkers of these persistent organic pollutants.

* To continue informing the public as an important factor towards nature conservation.

Marine mammals.

Efforts to obtain a larger number of individuals have already proved to be successsful. A first extension of the MARIN group intervention area outside the Belgian borders towards the Northern French coastline occured in 1995 . More recently, agreements to collaborate were taken within the framework of the European Programme BIOCET, which further extends the intervention area southwards to The Havre.

International contacts have been fruitful over the past years, the MARIN group multidisciplinary approach being of particular interest to other scientific teams. These international contacts recently led the MARIN group to organize the 2002 Annual

Conference of the European Cetacean Society (ECS) with the main topic being :'Marine mammals health : from individuals to populations' which will be held at the University of Liège.

<u>The MARIN group recommend :</u>

- To continue the efforts to obtain bycaught individuals in the future. Such individuals are of high interest as reference samples.

- Contacts with volunteers and national authorities is very positive but need to be continued.

- A continued monitoring of the health status of the populations is necessary.

- Enlarge the scope of a new scientific approach with the use of novel techniques would be useful. Of particular interest is the detection of specific biomarkers of POPs exposure.

- Results obtained during this research programme are important in the preparation of new legislation to improve both the already existing protection of the animals but also their indirect protection through the adaptation of human activities.

- Continue to operate the existing tissues bank for both seabirds and marine mammals. Such samples are of high interest to other scientific teams and can therefore be shared.

- To continue informing the general public as an important factor towards nature conservation.

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Annex.

List of species cited in the text.

Scientific name	English name
Sprattus sprattus	Sprat
Mallotus villosus	Capelin
Clupea harengus	Herring
Ammodytes sp.	Sandeel
Uria aalge	Common guillemot
Uria lomvia	Brünnich guillemot
Cepphus grylle	Black guillemot
Alca torda	Razorbill
Fratercula arctica	Atlantic puffin
Fulmarus glacialis	Fulmar
Hydrobates pelagicus	Storm petrel
Macronectes halli	Giant petrel
Rissa tridactyla	Kittiwakes
Larus argentatus	Herring gull
Larus ridibundus	Black-headed gull
Somateria mollissima	Common eider
Gavia immer	Common loon
Puffinus pufinus	Manx shearwater
Calonectris diomedea	Cory's shearwater
Catharacta skua	Great skua
Diomedea chrysostoma	Grey-headed albatross
Casmerodius albus	Common egret
Haematopus ostralegus	Oyster catcher
Podicep cristatus	Great crested grebe
Podicep nigricollis	Eared grebe
Sula bassana	Gannet
Calidris alba	Sanderling
Ardea cinerea	Grey heron
Sturnus vulgaris	Starling
Tyto alba guttata	Barn owl
Coturnix coturnix japonica	Common quail
Phocoena phocoena	Common porpoise

Lagenorhynchus albirostris	White-beaked dolphin
Leucopterus acutus	Wite-sided dolphin
Stenella coeruleoalba	Striped dolphin
Physeter macrocephalus	Sperm whale
Balaenoptera physalus	Fin whale
Balaenoptera acuturostrata	Minke whale
Balaena mysticetus	Right whale
Eschrichtius robustus	Grey whale
Mesoplodon bidens	Sowerby's beaked whale
Phoca vitulina	Harbour seal
Halichoerus grypus	Grey seal
Cystophora cristata	Hooded seal
Phoca hispida	Ringed seal

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