

Impulse Programme in Marine Sciences  
The Prime minister's Services  
Office for Scientific, Technical and Cultural Affairs

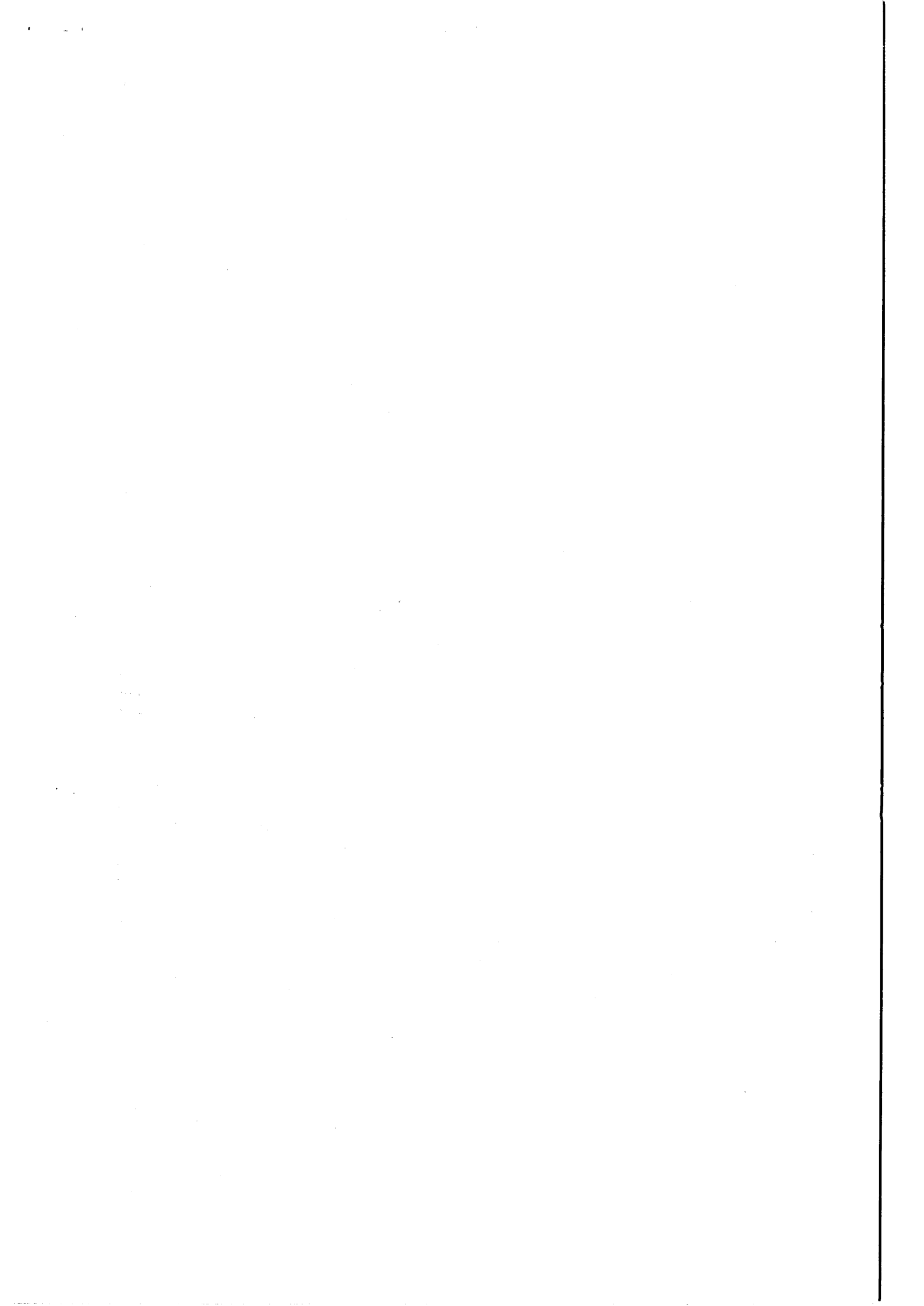
Contracts MS MS/03/031, MS/12/032 & MS/12/033.

**Pathology and ecotoxicology of seabirds  
and marine mammals  
of the North Sea and adjacent areas.**  
(October 1992 - December 1996).

Coordination report  
by  
Claude R. Joiris and Ludo Holsbeek.

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

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## **Pathology and ecotoxicology of seabirds and marine mammals of the North Sea and adjacent areas.**

Coordination report  
CR Joiris & L Holsbeek

- Service of Anatomy and Pathology, Veterinary Medicine, Liège University (Prof Coignoul) (MS/12/033);
- Service of Oceanology, Liège University (Prof Bouquegneau) (MS/12/032);
- Laboratory for Ecotoxicology and Polar Ecology, University of Brussels (Prof Joiris, coordinator) (MS/03/031).

### **Abstract.**

The research project covers the pathological and ecotoxicological study of seabirds and marine mammals beached along the Belgian North Sea coast, from October 1992 to December 1996.

The objectives were to identify the cause of death after anatomo-pathological and ecotoxicological analyses and indirectly, to evaluate the state of health of the populations involved. A second objective was to investigate whether or not a link could be established between the general degree of pollution in the southern North Sea and stable contaminant levels in top predators. As a third objective, the mechanisms for accumulation, excretion and detoxification in marine top predators were given a closer examination.

A total of 553 birds (28 species) and 17 marine (6 species) were passed through the Belgian recovery scheme for anatomo-pathological research. A total of 287 birds and 16 marine mammals were analyzed for stable trace elements (three tissues each), and a selected subsample was treated for analysis of selenium and metallothioneins, both involved in separate detoxification mechanisms.

**Seabirds.** In an early stage of the research, the common guillemot *Uria aalge*, which outnumbered by far all other species, was chosen as our target species. The main problems potentially affecting the health of the population wintering in the southern North Sea showed to be oiling, low total body weight and cachexia, gastric hemorrhages and elevated Cd, MeHg and PCB concentrations.

Oiling is still the major cause of death for wintering seabirds in the southern North Sea, although decreasing with time as a result of increased surveillance. No major concern about bacteriological or viral infections is to be raised. Stable pollutant levels, however, might play an important role in a multifactorial process leading to debilitation and death of the animals: considerably high levels of Cu, Zn, Cd, MeHg and PCBs are reasons for concern. No major case of mass mortality was reported. The general state of health at the population level is poor (oiled birds = control): a large majority of individuals is to a high degree cachectic with a low body weight and relatively high levels of stable micropollutants.

The link that was established between an increase of stable pollutant levels and the duration of the stay in the southern North Sea clearly responds to the second objective of this study. The general degree of pollution in the southern North Sea (compared to summer resident areas) has a direct and immediate impact on tissue contaminant levels of seabirds. Pollutant levels at the end of the wintering period thereby reach concentrations described as lethal.

**Marine mammals.** As foreseen, numbers of marine mammals collected along the Belgian coast were too low to allow a separate scientific interpretation: they must be integrated in broader studies at the level of the whole North Sea and adjacent areas.

The main conclusion, after normalization for age (length) and sex is that our contamination data can be integrated in results from the other regions: no contamination problem was detected, to be interpreted as acute cases directly bound to local conditions

in the southern North Sea. This includes the sperm whales beached in Belgium and the Netherlands.

High levels of Cd, Hg and organochlorines were found. Since marine mammals, by their long life span and their long displacements, integrate contamination on large temporal and geographical scales, such contaminations might affect large populations on a large range and thus reflect important pollution effects.

Selenium and metallothioneins play an important role in the detoxification of heavy metals in seabirds and marine mammals. Very high levels of selenium in the liver of marine mammals, linked to equally high levels of inorganic Hg indicate an adequate elimination of MeHg. As for the binding of non-essential metals to the metallothionein proteins, large fractions (Cd, Cu) were found to be in a free state and might reach toxic levels in some individuals involved.

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## **Introduction.**

This report is based on yearly reports and a synthetic report produced by the 3 teams involved in the Impulse Programme in Marine Sciences funded by the Prime Minister's Services, Office for Scientific, Technical and Cultural Affairs:

- Service of Anatomy and Pathology, Veterinary Medicine, Liège University (Prof Coignoul) (MS/12/033);
- Service of Oceanology, Liège University (Prof Bouquegneau) (MS/12/032);
- Laboratory for Ecotoxicology and Polar Ecology, University of Brussels (Prof Joiris, coordinator) (MS/03/031).

It covers the pathological and ecotoxicological study of seabirds and marine mammals found along the Belgian coast of the North Sea from October 1992 to December 1996. Detailed information and basic results are to be found in these original reports, and were thus not repeated here.

## **Objectives and scientific tasks**

The objectives of the research project were threefold. First, to identify the cause of death after anatomo-pathological and ecotoxicological analyses of the animals found dead and indirectly, to evaluate the state of health of the populations involved. A second objective was to investigate whether or not a link could be established between the general degree of pollution in the southern North Sea and stable contaminant levels in top predators. As a third objective, the mechanisms for accumulation, excretion and detoxification in marine top predators were given a closer examination. For these purposes, 200 dead animals were planned to be

collected yearly from the beach for anatomo-pathological research. Three tissues of 70 selected specimens were to be further analyzed for ecotoxicology (heavy metals, organochlorines and detoxification mechanisms). In the case of seabirds, samples were to be regularly collected during the at sea wintering period and in cases of sudden mass mortality. Because of low numbers involved, all marine mammals were to be treated with priority.

As could be foreseen, bird samples outnumbered by far the number of marine mammals at our disposal. Large scale scientific conclusions are likewise linked to the statistically significant analyses of seabird.

A total of 553 birds, belonging to 28 species, and 17 marine mammals of 6 species, were passed through the Belgian recovery scheme under a shared coordination of the Federal Mathematical Model for the North Sea (BMM), the Flemish Institute for Nature Protection and the royal Belgian Institute for Natural Sciences for anatomo-pathological research. A total of 287 birds and 16 marine mammals (three tissues when available) were analyzed for stable trace elements, a selected subsample was treated for analysis of selenium and metallothioneins, both involved in separate detoxification mechanisms.

Yearly, 3 to 5 general meetings of the task group were scheduled; additionally, both teams involved in the ecotoxicological study had 3 to 5 more working sessions in order to coordinate their scientific efforts. As foreseen, scientific data for all different types of analysis were passed each year into the ICES Environmental Data Reporting framework.

## **Results and discussion.**

### **1. Seabirds.**

The study of seabirds mainly concerned the most abundant species encountered, and represented in the sampling during the winter: the common guillemot *Uria aalge*. Some other species were analyzed as well, but in much lower numbers, and will not be discussed here.

Due to the existence of a significantly lower contamination by Hg, Zn, Fe and organochlorines of the individuals found dead on the beach compared to the ones found alive and eventually dying later on in rehabilitation centres, it was decided to exclude the last ones and only include the ones found dead on the beach, since this increased contamination might represent an artifact due for instance to the food given in the centres (table 1; Fig. 1).



Table 1: Total body weight (g) and trace elements concentration ( $\mu\text{g}/\text{g dw}$ ) in tissues of common guillemots *Uria aalge* beached along the Belgian coast, either found dead ("beach") or after stay in a rehabilitation centre ("centre"); mean values; nd: not determined; differences significant at the  $p < 0.01$  level are shown as boxes.

	Beach		Centre		p =				
Body mass	725 ± 125 700 440-1180 (168)	634 ± 102 600 465-1280 (164)			< 0.01				
	Liver		Kidney		Muscle				
	Beach	Centre	p =	Beach	Centre	p =			
Cu	52 ± 17 52 14 - 100 (144)	51 ± 26 50 10 - 152 (104)	ns	28 ± 12 27 1.1 - 76.3 (110)	31 ± 13 31 2 - 74 (53)	ns	18 ± 6 18 9 - 53 (145)	20 ± 11 18 3 - 90 (107)	ns
Zn	145 ± 39 138 66 - 328 (144)	168 ± 46 158 84 - 413 (104)	< 0.01	169 ± 41 168 41 - 284 (111)	173 ± 46 176 37 - 286 (54)	ns	60 ± 14 59 31 - 131 (145)	73 ± 31 67 36 - 235 (107)	< 0.01
Fe	2549 ± 1354 2274 393 - 5928 (144)	3557 ± 1564 3468 775 - 7946 (104)	< 0.01	613 ± 294 569 122 - 2376 (111)	700 ± 257 668 367 - 1759 (53)	< 0.05	669 ± 241 641 337 - 2428 (145)	903 ± 784 732 78 - 5724 (107)	< 0.01
Cd	2.4 ± 1.6 2.1 <dl - 10.1 (144)	2.5 ± 1.9 2.1 <dl - 9.7 (104)	ns	7.8 ± 6.5 6.4 <dl - 39.9 (111)	6.2 ± 5.3 4.7 <dl - 30.2 (54)	ns	<dl	<dl	
Total Hg	5.9 ± 2.9 5,4 0.8 - 20.7 (156)	7.9 ± 6.3 6,0 1.2 - 35.8 (125)	< 0.05	4.6 ± 3.0 4,0 1.0 - 23.8 (90)	5.7 ± 2.4 4,5 4.4 - 9.3 (4)	ns	2.1 ± 1.1 1,8 0.3 - 6.7 (163)	3.8 ± 3.4 2,8 0.4 - 23.2 (139)	< 0.01
Org. Hg	4.6 ± 2.2 4,1 0.8 - 14.1 (138)	6.6 ± 5.4 5,0 1.3 - 32.3 (105)	< 0.01	3.3 ± 1.5 3,0 1.0 - 6.9 (55)	5.2 ± 1.8 4,6 3.6 - 7.8 (4)	< 0.05	1.6 ± 0.8 1,4 0.3 - 4.9 (136)	3.1 ± 2.8 2,2 0.4 - 17.8 (114)	< 0.01
Inorg. Hg	1.1 ± 1.1 0,9 0.0 - 6.5 (135)	1.3 ± 1.6 0,9 0.0 - 10.8 (105)	ns	0.9 ± 0.7 0,7 0.0 - 2.6 (54)	0.6 ± 0.7 0,5 0.0 - 1.5 (4)	ns	0.4 ± 0.4 0,3 0.0 - 1.9 (136)	0.6 ± 0.8 0,4 0.0 - 5.3 (113)	ns
Sum PCB	5.7 ± 6.0 3,5 0.3 - 27.2 (130)	11.7 ± 13.0 8,7 1.0 - 60.4 (68)	< 0.01	3.4 ± 2.8 2,6 0.1 - 12.8 (88)	2.6 ± 1.6 2,2 1.1 - 4.7 (4)	ns	2.1 ± 1.8 1,6 0.1 - 10.5 (130)	5.4 ± 10.0 3,0 0.2 - 81.9 (77)	< 0.01
Total lipids	0.18 ± 0.07 0,17 0.03 - 0.60 (120)	0.16 ± 0.07 0,15 0.06 - 0.33 (32)	ns	nd	nd		0.10 ± 0.08 0,08 0.01 - 0.63 (119)	0.14 ± 0.12 0,10 0.05 - 0.65 (31)	< 0.01
Polar lipids	0.11 ± 0.03 0,11 0.04 - 0.29 (130)	0.11 ± 0.04 0,10 0.07 - 0.27 (68)	ns	0.12 ± 0.02 0,12 0.03 - 0.17 (88)	0.07 ± 0.04 0,08 0.01 - 0.11 (4)	< 0.05	0.04 ± 0.03 0,03 0.01 - 0.16 (130)	0.05 ± 0.04 0,04 0.01 - 0.19 (77)	ns

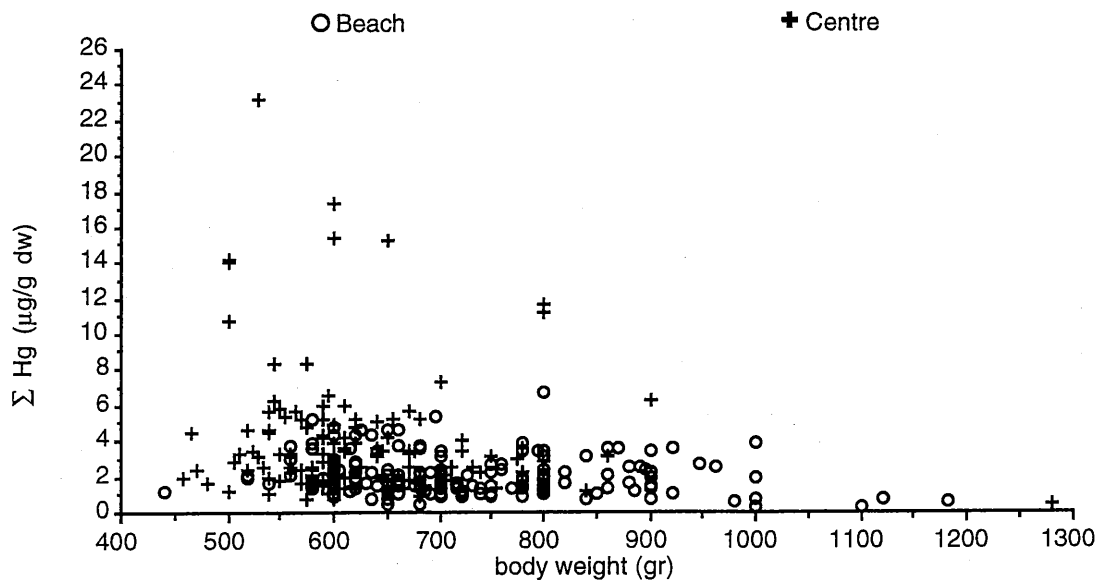


Fig. 1. Mercury concentration in common guillemots *Uria aalge* beached on the Belgian coast: total Hg in muscle (n = 298). Circles: found dead; crosses: beached alive, dead in rehabilitation centres. Similar data are available for  $\Sigma$ Hg, PCBs and pesticides (DDE) in muscle and liver (Joiris et al., 1997).

### 1. 1. Total body weight.

The most obvious observation concerns the low total body weight of the guillemots wintering in the southern North Sea: 700 g instead of the normal summer weight of 1 kg.

Most striking is that this low weight is already observed on the first individuals found in November - December: during winter, the weight is not decreasing any longer (Fig. 2), nor is the lipid content (Fig. 3). So that the simple hypothesis of a starvation during winter does not seem to provide the real explanation. Moreover, the fact that contamination is increasing during winter (see further) implies that the guillemots are actually feeding in the southern North Sea. Since very few "heavy" individuals only were found in November - December (Figs. 2 & 3), this phenomenon might concern the whole wintering population: among the animals collected, at least some were heavily oiled and are considered as having died rapidly: they should reflect the "normal" weight of the population. In our samples, the weight of the oiled birds was only slightly higher than the ones without any oiling (747 and 698 g respectively,  $p = 0.02$ ), so that the weight of the at sea population could be 750 g, i.e. 25% lower than the summer weight. This provides a maximal value, since total body weight includes the weight of oil on the feathers.

Moreover, the weight of the animals found with full stomach, and thus actively feeding shortly before their dead, was not higher either.

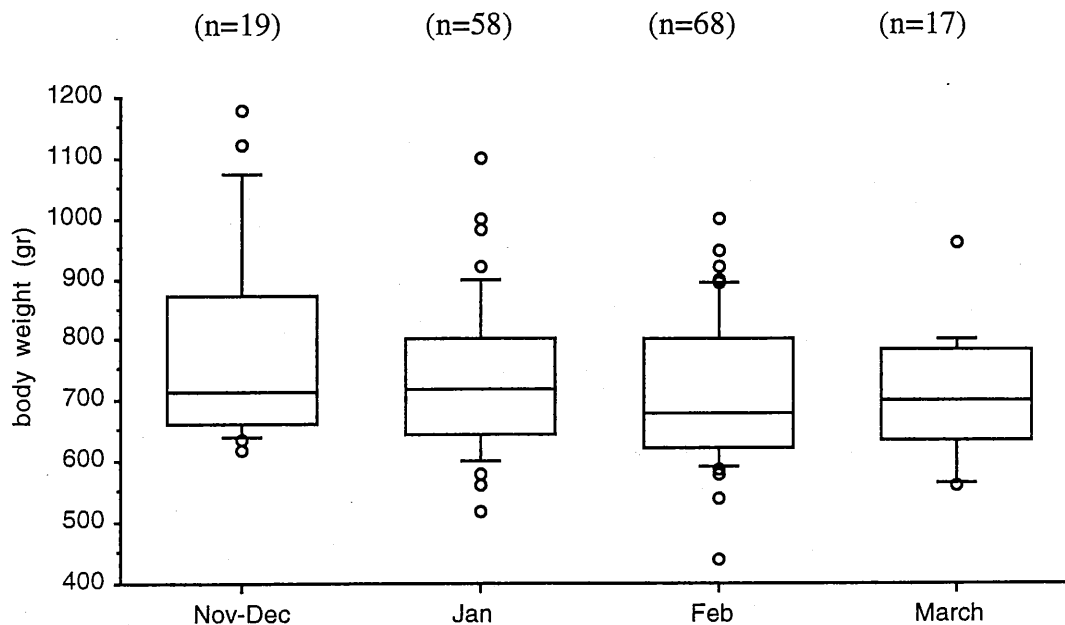


Fig. 2. Total body weight of beached common guillemots as a function of collection date. In the boxes: median (50%) value, 75%, 90%, outsiders; n = number of birds; p = 0.3 (Joiris et al., 1997).

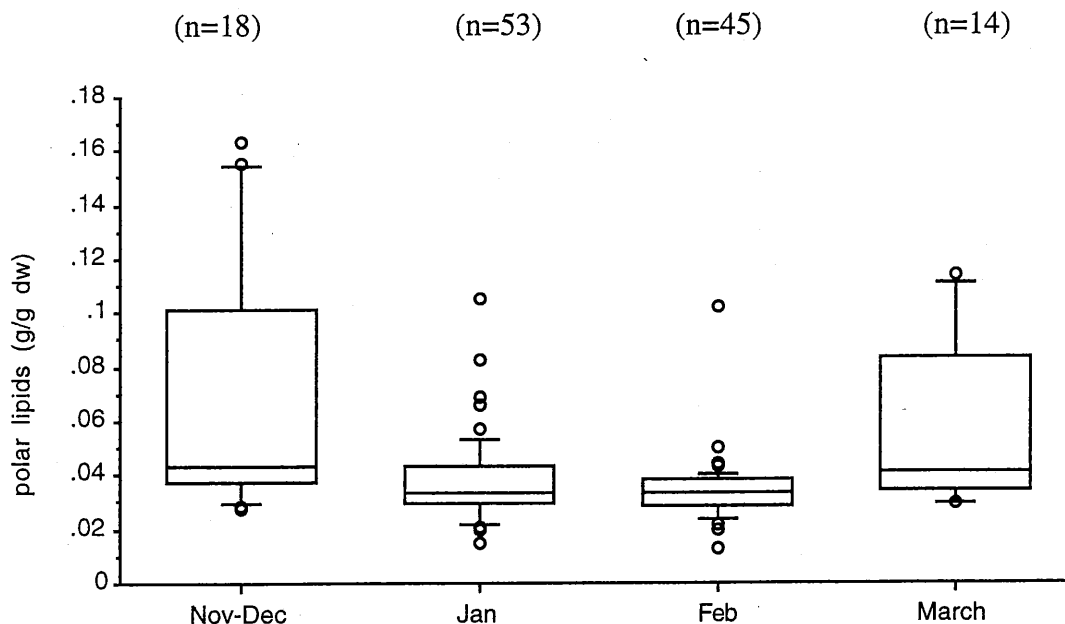


Fig. 3. Polar lipids content in the muscle of common guillemots as a function of collection date (g/ g dw). Similar data are available for liver and kidney. (Joiris et al., 1997).

## 1. 2. Oiling.

More than half of the guillemots were oiled (55%), some of them both externally and internally. This makes oiling the first cause of mortality, especially by adults (69% oiled), while other causes play an important role for the juveniles, as expected ("natural" mortality) (51% oiled). On

the longer term, however, the proportion of oiled birds decreased: it used to be much higher and reached 80% in the sixties (Offringa et al., 1995).

This decreasing proportion of oiled seabirds can be interpreted as reflecting a lower oil pollution in the North Sea, e. g. due to strict legislations in the early seventies and an important air born surveillance of the ships releasing oil (Schallier et al., 1996).

### 1. 3. Pathology.

The 2 main observations concern the high proportion of cachectic birds (see higher: weight), and the occurrence of gastro - enteropathy (Jauniaux et al., 1997). Different hypotheses can be proposed in order to explain the links between cachexy, hemorrhagies and contamination, but no clearcut mechanism could definitively be proposed yet. More analyses are necessary, with special attention to the comparison between "early" and "late" mortalities. This will not only help elucidating the mechanisms involved, but also indentifying which phenomenons depend on the stay of the birds in the southern North Sea, and which ones affect the birds on a much larger geographic zone.

In future, the study of summer guillemots from Scotland and Norway, as well, if possible, as winter birds from the same regions, could provide the necessary information and allow indentifying the mechanisms involved and the period when they affect the seabirds. This aspect will be developped thanks to collaboration with Dr Furness (Scotland) and Dr Barrett (Norway). This collaboration concerns contamination as well (see further).

### 1. 4. Contaminants.

Among the micropollutants, 3 seem to deserve special attention:

- Cd levels are very high, compared to literature data and might have lethal effects. Moreover, the Cd to Zn ratio is abnormally high as well, confirming the existence of contamination problems (Debacker et al., 1997). It is however necessary to study also the speciation of the heavy metals, in order to understand their potential toxicity: in the case of heavy metals, the presence of metallothioneins fixing a significant proportion of the residues (Bouquegneau et al., 1996) can be considered as a form of detoxification.

- concerning the organic contaminants, Hg (mainly organic MeHg) and organochlorines (mainly PCBs, but also DDTs) show high values and might cause trouble as well (Debacker et al., 1997). A more detailed analysis of organic contaminants (Joiris et al., 1997) showed that winter concentrations are much higher than the summer ones, and are increasing during winter in the southern North Sea. Since total body weight nor lipid content are decreasing during winter (see higher), this phenomenon does

not reflect an effect of lipid consumption causing a remobilization of lipophilic residues and an increased concentration, but a real increase of total body burden. Combining the summer data collected in Scotland (Stewart et al., 1994) and our winter data, we established a yearly cycle of Hg contamination (Fig. 4). All results can be explained by a simple uptake - excretion model with a turn-over (half life) of about 1 month, reflecting the consumption of prey with a markedly higher contamination in winter in the southern North Sea than in summer in north-east Atlantic water.

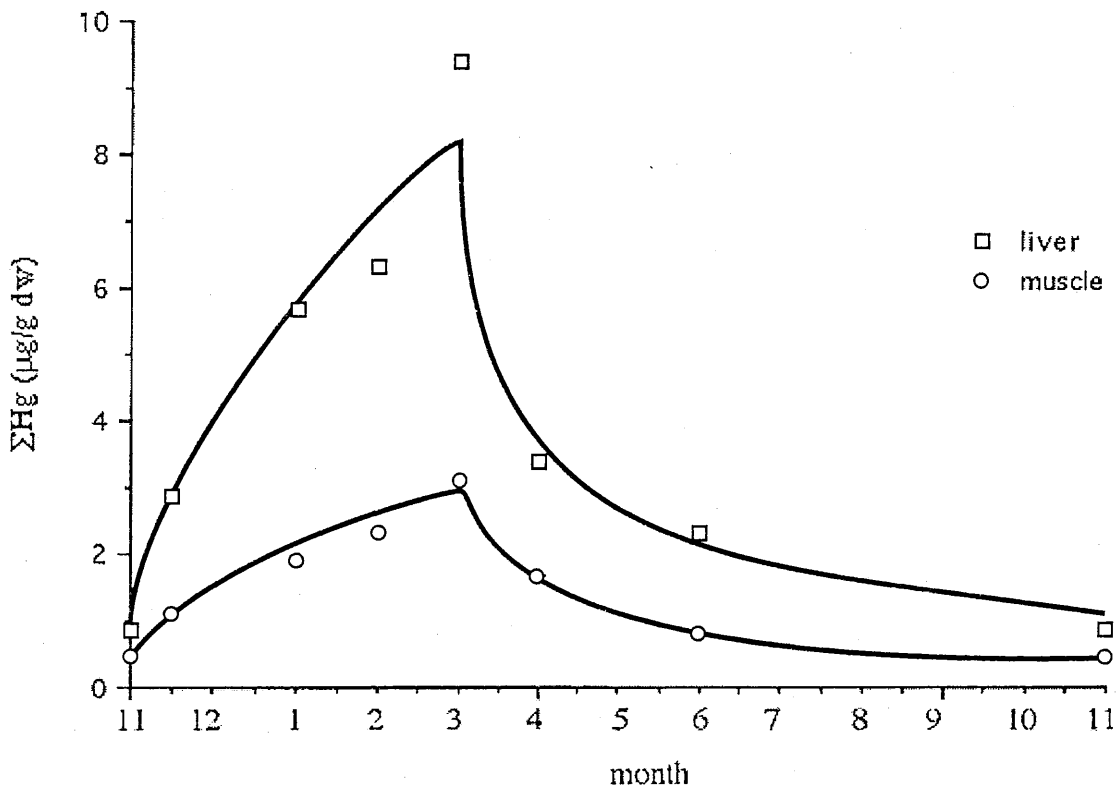


Fig. 4. Annual cycle of Hg concentration in liver and muscle of the common guillemot. April - November: Stewart et al., 1994; mid-November - March: Joiris et al., 1997. Curve fitted by eye (Joiris et al., 1997).

An important consequence is that data must be normalized for collection date before allowing any geographical or temporal comparison: early winter birds still show a contamination close to the summer ones. Late winter birds must be considered as reflecting the southern North Sea levels, if they are to be used as bioindicators. In this frame, the conclusion is that the food consumed by guillemots in the southern North Sea ecosystem is much more contaminated by organic micropollutants - MeHg and PCBs - than in Atlantic water off Scotland.

During winter, guillemots reach median MeHg and PCB levels of 10 ppm dw in the liver, generally considered as causing lethal effects - a few individuals even reaching twice this concentration! These concentrations could still be significantly higher, since the plateau values are not actually reached during winter (Fig. 4): if the guillemots were staying longer in the southern North Sea, their contamination could even be higher.

A comparison with literature data (table 2) shows that other metal concentrations also show clearly higher winter values in the southern North Sea: Cu, Zn, Cd. They might have a yearly cycle like the one established for Hg, but more data are necessary in order to confirm this hypothesis. On the other hand, earlier determinations of Hg concentration in guillemots (Delbeke et al., 1984) do not reflect any obvious decrease during the last 20 years: they confirm the values detected in this study.

In order to confirm these conclusions, actual total body burdens are now being established, by multiplying concentrations by the total weight of the main tissues. In future, it would be very interesting to realize a similar exercise with guillemots collected during summer in Scotland and Norway (see higher: weight).

## 1.5. Conclusion.

The data collected during this study allowed us to identify the main problems potentially affecting the health of the population wintering in the southern North Sea: low total body weight, oiling, cachexy, gastric hemorrhages, high Cd, MeHg and organochlorine concentrations.

Different mechanisms for establishing the link between these phenomena could be proposed, but none of them with enough certainty to actually identify the main one(s):

- cachexy might be due to starvation, but the animals are already very meager when arriving in the southern North Sea in November - December. On the other hand, they must be feeding since their contamination increases during winter, due to the consumption of prey with a higher contamination than in summer. Finally, it is difficult to understand why guillemots should stay in the southern North Sea, if food was so scarce there that they could starve: they easily could go a bit further south, and for instance reach Atlantic water in the Channel.

Table 2: Heavy metals concentration ( $\mu\text{g/g dw}$ ) in tissues of the common guillemot *Uria aalge* of different origin (mean values); nd: no determination.

Tissue	place	period	n	Cu	Zn	Cd	$\Sigma\text{Hg}$	reference	
liver	Belgian coast	winter 1970 - 1981	51	nd	nd	nd	7.2	Delbeke et al., 1984	
		winter 1992 - 1995	143	52	145	2.4	6.1	this study: Debacker et al., 1997	
	NW Scotland	summer 1988	83	16	65	1.8	2.4	Stewart et al., 1994	
		summer 1992 - 1993	10	20	87	3.1	1.9	Wenzel & Gabrielsen, 1994	
	kidney	Belgian coast	winter 1970 - 1981	51	nd	nd	nd	4.4	Delbeke et al., 1984
			winter 1992 - 1995	143	28	169	7.8	4.6	this study: Debacker et al., 1997
NW Scotland		summer 1988	83	14	74	8.2	2.6	Stewart et al., 1994	
		summer 1992 - 1993	10	20	87	3.1	1.9	Wenzel & Gabrielsen, 1994	
muscle	Belgian coast	winter 1992 - 1995	143	28	169	7.8	4.6	this study: Debacker et al., 1997	
		summer 1988	83	12	25	nd	1.0	Stewart et al., 1994	
	N Norway	summer 1992 - 1993	10	20	87	3.1	1.9	Wenzel & Gabrielsen, 1994	
		summer 1992 - 1993	10	20	87	3.1	1.9	Wenzel & Gabrielsen, 1994	

- gastric hemorrhagy could be due to oiling or "starvation", but the link between both is not clear: oiled birds do not show more cachexy than the non-oiled ones, on the contrary (see higher); cachectic individuals, on the other hand, show a higher occurrence of gastric hemorrhagy (69 and 31% respectively).

- for micropollutants, the situation is clear for MeHg and the organochlorines: their concentration and total body load are increasing during winter, and reach lethal levels at the end of the winter. This, however, cannot explain the early winter mortality, since mortality is following the arrival of the guillemots on their winter grounds, possibly with a lag between the peaks values of numbers at sea and of beached animals, but without a massive increase of mortality at the end of winter (see Debacker et al., 1997). A complementary study is necessary, in order to define if the problems of high Cd contamination follow a similar pattern, the Cd concentration being high enough to have caused cachexy.

## **2. Marine mammals.**

As foreseen, numbers of marine mammals collected along the Belgian coast were too low to allow a separate scientific interpretation: they must be integrated in broader studies at the level of the whole North Sea and adjacent areas.

The main conclusion, after normalization for age (length) and sex is that our contamination data can be integrated in results from the other regions: no contamination problem was detected, to be interpreted as acute cases directly bound to local conditions in the southern North Sea. This includes the sperm whales beached in Belgium and the Netherlands.

We were however able to detect problems of high contamination by Cd, Hg and organochlorines. Since marine mammals, by their long life span and their long displacements, integrate contamination on large temporal and geographical scales, such contaminations might affect large populations on a large range - for sperm whales, the whole NE Atlantic - and so reflect very important pollution effects.

This why in future, the international collaboration and coordination will continue to increase, and the Belgian data incorporated in much broader studies. As it is now, we already developed close collaboration with Germany (both the Baltic and North Sea populations of the harbour porpoise), the Netherlands (Hg in harbour porpoise), Ireland (Hg in dolphins) and France (Hg in dolphins)(Joiris et al., Holsbeek et al., Siebert et al., in prep.), as well as in Black Sea dolphins and porpoises (Hg, organochlorines: Copernicus contract by the European Commission).



### 3. General conclusions

A first objective of the project was to establish a cause of death and to provide a general view about the state of health at a population level. Because of the need for statistically significant numbers, general conclusions are based on the analysis of the guillemot mainly.

First conclusion is that oiling is still the major cause of death for wintering seabirds in the southern North Sea, although decreasing with time as a result of increased surveillance. At a second level, no major concern about bacteriological or viral infections is to be raised. Stable pollutants levels, however, might play an important role in a multifactorial process leading to debilitation and death of the animals: considerably high levels of Cu, Zn, Cd, MeHg and PCBs are reasons for concern. No major case of mass mortality was reported. If our sample is to be regarded as a normal population sampling - as can be concluded -, the state of health at the population level is poor: individuals are to a high degree cachectic with a low body weight and relatively high levels of stable micropollutants.

The link that was established between an increase of stable pollutant levels following a longer stay in the southern North Sea clearly responds to the second objective of this study. The general degree of pollution in the southern North Sea (compared to summer resident areas) has a direct and immediate impact on tissue contaminant levels of seabirds. Pollutant levels at the end of the wintering period thereby reach levels close to the ones described as lethal in literature.

Selenium and metallothioneins play an important role in the detoxification of heavy metals. Very high levels of selenium in the liver of marine mammals, linked to equally high levels of inorganic Hg reflect an important demethylation of MeHg in time (Joiris et al., 1991). As for the binding of heavy metals to the metallothionein proteins, large fractions (50 % of Cd in marine mammals; 45 to 75 % for Cu, Zn and Cd for seabirds) were found to be not bound to metallothioneins and might thus have reached harmful levels in the individuals involved.

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