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Etude pathologique et écotoxicologique des oiseaux et des mammifères marins dans la mer du Nord et les régions avoisinantes.

RAPPORT DE SYNTHESE

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par

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Table des matières

1. Introduction

2. Les oiseaux marins

2.1. Répartition des espèces.

  2.1.1. Choix de l'espèce: le guillemot de Troïl (*Uria aalge*).

2.2. Les oiseaux récoltés sur les plages.

2.3. Les oiseaux soignés en centre de revalidation.

2.4. Analyses réalisées.

  2.4.1. Analyses en métaux lourds.

  2.4.2. Analyses des lipides.

  2.4.3. Détection des métallothionéines.

2.5. Analyse de six hivers successifs.

2.6. Rôle des métallothionéines.

2.7. Autres espèces analysées.

2.8. Références.

3. Les mammifères marins

3.1. Répartition des espèces.

3.2. Etude détaillée: échouage de quatre cachalots (*Physeter macrocephalus*), Koksijde, 1994.

3.3. Références.

4. Liste des travaux réalisés dans le cadre du contrat

4.1.Articles.

4.2. Rapports pour colloques.

4.3. Mémoires.

4.4. Posters.

1. Introduction

Ce rapport de synthèse reprend l'ensemble des résultats des analyses effectuées sur des oiseaux et des mammifères marins récoltés à la côte belge depuis l'hiver 1992-1993 jusqu'à l'hiver 1995-1996. Les oiseaux provenaient d'une part des centres de revalidation de la côte (Oostende, Blankenberge, Het Zwin, Nieuwpoort...) et, d'autre part des plages, où ils sont régulièrement ramassés grâce à une collaboration avec l'Instituut voor Natuurbehoud de Bruxelles (P. Meire, H. Offringa). Les mammifères marins étaient soit trouvés échoués sur les plages, soit capturés accidentellement dans les filets de pêche et mis à la disposition du réseau d'intervention. L'ensemble des individus, oiseaux et mammifères marins, ont été autopsiés et disséqués par l'équipe vétérinaire du Service d'Anatomie Pathologique du professeur F. Coignoul (Université de Liège). L'ensemble des échantillons prélevés était alors transmis d'une part au Laboratoire d'Ecotoxicologie et d'Ecologie Polaire du professeur C. Joiris (Vrije Universiteit van Brussels) et, d'autre part, vers notre équipe, le Laboratoire d'Océanologie du professeur J-M. Bouquegneau (Université de Liège).

2. Les oiseaux marins

2.1 Répartition des espèces.

Sur l'ensemble des hivers considérés, un total de 193 oiseaux ont été analysés appartenant à 13 espèces différentes. Parmi celles-ci, le guillemot de Troïl *Uria aalge* se retrouve en nombre (n = 161 individus), le reste de l'effectif (n = 32) étant représenté par des oiseaux appartenant à 13 espèces différentes.

Espèces	Effectifs (n =)
Uria aalge (guillemot de Troïl)	161
Alca torda (petit pinguin)	4
Fulmarus glacialis (pétrel fulmar)	2

Rissa tridactyla (mouette tridactyle)	4
Larus ridibundus (mouette rieuse)	3
Larus argentatus (goéland argenté)	6
Larus fuscus (goéland brun)	1
Larus canus (goéland marin)	2
Larus sp. (laridé non déterminé)	1
Melanitta nigra (macreuse noire)	5
Gavia arctica (plongeon arctique)	1
Gavia stellata (plongeon catmarin)	1
Fulica atra (foulque macroule)	1
Podiceps cristatus (grèbe huppé)	1
TOTAL	**193**

2.1.1. Choix de l'espèce: le guillemot de Troïl.

L'ensemble des analyses effectuées concerne principalement le guillemot de Troïl (*Uria aalge*): espèce pélagique occupant un niveau trophique élevé, il est largement distribué en Mer du Nord, ne migre pas sur de longues distances et s'échoue en nombre important sur nos côtes pendant l'hiver.

Il est considéré comme un bon indicateur des niveaux de contamination du milieu et a été utilisé comme tel dans différentes études scientifiques (Osborn *et al.*, 1984; Stewart, 1994; Stewart *et al.*, 1994; Stewart *et al.*, 1996;Wenzel & Gabrielsen, 1995;Wenzel & Adelung, 1996)

2.2. Les oiseaux récoltés morts sur les plages.

Les oiseaux échoués morts sur le littoral belge sont régulièrement collectés par l'équipe de l'Instituut voor Natuurbehoud. Ces derniers assurent d'une part une collecte hebdomadaire le long du tronçon situé entre Oostende et Nieuwpoort, et d'autre part une collecte mensuelle sur l'ensemble du littoral belge.

2.3. Les oiseaux soignés en centre de réhabilitation.

Les oiseaux échoués vivants sont dirigés vers les différents centres de réhabillitation de la côte où ils sont généralement nettoyés des souillures d'hydrocarbures dont ils sont les victimes et subissent un traitement plus ou moins long avant d'être relachés. Il faut néanmoins savoir que les taux de réussite de réhabilitation d'oiseaux sont très faibles avec un taux de survie très bas (Sharp, 1996). La plupart des oiseaux envoyés vers les centres de réhabilitation y meurent. De par ces différentes interventions sur ces individus (traitements, durée du séjour inconnue), les oiseaux provenant des centres de réhabilitation n'ont plus fait l'objet d'analyses toxicologiques à partir de l'hiver 1993-1994. L'analyse de leurs niveaux de contamination a également montré que des différences statistiquement significatives existaient entre les individus « plage » et ceux provenant des centres de revalidation , ces derniers présentant des niveaux de contamination plus élevé en Zn, Fe, Hg total et PcBs. De plus, l'étude des rapports isotopiques $\delta^{13}C$ et $\delta^{15}N$ dans les muscles pectoraux et les foies des oiseaux ayant séjourné dans un centre de revalidation, met également en évidence une différence de régime alimentaire par rapport aux oiseaux trouvés morts sur nos plages. Pour toutes ces raisons, les oiseaux provenant des centres de revalidation ne sont pas comparables aux individus directement récoltés morts sur les plages et représentent une source d'erreur potentielle pour l'interprétation des résultats. Ils ont donc été systématiquement éliminés et ce à partir de l'hiver 1993-1994.

2.4. Analyses réalisées.

2.4.1. Analyse en métaux lourds.

Après avoir été pesés et sechés pendant 48 heures à l'étuve (100°C) pour obtenir le poids sec, les échantillons (foies, reins, muscles) sont placés au bain-marie (températures croissantes de 20 à 90°C) et additionnés d'une solution mixte d'acide nitrique concentré et d'acide chloridrique (3:1;v:v). Une fois minéralisés, les échantillons sont dilués, filtrés et les contenus en métaux lourds (Cu, Zn, Fe, Cd, Cr, Ni, Pb) analysés par spectrophotométrie d'absorption atomique. Parallèlement à l'analyse des échantillons, du matériel certifié (CRM 278 Community Bureau of Reference, Commission of the European Communities) a également été analysé pour vérifier la qualité des analyses effectuées. On retrouve de 92 à 102 % pour le Cu, Zn et Fe et 80 % pour le Cd. Les limites de détections sont de 0.01 g/g poids sec pour le Cu, 0.33 g/g poids sec pour le Zn et 0.22 g/g poids sec pour le Cd.

2.4.2. Analyse des lipides.

La méthode d'extraction des lipides est celle mise au point par Barnes et Blackstock (1973).

2.4.3. Détection des métallothionéines.

Environ 2 gr de poids frais de foie ou de rein sont homogénéisés (Ultra Turrax 18/10) dans deux volumes d'une solution de formiate amonique 0.01M (pH = 7.4) contenant une solution 10mM de dithiothréitol (antioxydant) et centrifugé à 26000 g pendant deux heures. Le surnageant est alors déposé sur une colonne de chromatographie (tamis moléculaire) contenant un Ultrogel AcA 54 (LKB). La présence des métallothionéines est détectée par lecture des fractions chromatographiques récoltées à 215 et 240 nm respectivement. Le contenu métallique (Cu, Zn, Cd) des fractions chromatographiques est également déterminé par absorption atomique à flamme.

2.5. Analyse de six hivers successifs.

La présente analyse (Annexe 1) est actuellement sous presse sous le titre: « Ecotoxicological and pathological studies of common guillemots *Uria aalge*, beached on the Belgian coast during six successive wintering periods (1989-90 to 1994-95) » dans la revue scientifique « Diseases of Aquatic Organisms ».

2.6. Rôle des métallothionéines.

La présente analyse (Annexe 2) a été publiée sous le titre: « Role of Metallothioneins in Metal Regulation by the Guillemot *Uria aalge* » dans la revue scientifique Comparative Biochemistry and Physiology (Volume 113C: 135-139, 1996).

2.7. Autres espèces analysées.

Parmi les autres espèces plus particulièrement analysées, on retrouve le petit pinguoin *Alca torda*, le pétrel fulmar *Fulmarus glacialis*, la mouette rieuse *Larus ridibundus* et le goéland argenté *Larus argnetatus*. Les résultats des analyses pour ces quatres espèces ont été comparés aux résultats obtenus pour le guillemot de Troïl *Uria aalge* en vue d'évaluer les variations des niveaux de contamination qui peuvent exister entre différentes espèces d'oiseaux fréquentant la Mer du Nord. Les principales conclusions de ce travail indiquent:

- des teneurs élevées en Cu et Zn des Alcidés (petit pinguoin et guillemot de Troïl) comparées aux données de la littérature;
- les teneurs en Cu des deux espèces d'Alcidés (petit pinguoin et guillemot de Troïl)dans les foies et les reins, sont toujours plus élevées que celles obtenues pour les trois autres espèces analysées;
- les teneurs en Cd du pétrel fulmar sont plus importantes que pour les autres espèces analysées, ce qui serait directement lié à son régime alimentaire (composé principalement de céphalopodes).

L'ensemble de ce travail a été réalisé dans le cadre d'une Maîtrise en Océanologie par Mlle A. Janosi en 1994-95.

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« Diseases of Aquatic Organisms », *in press.*

Annexe 1

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

V. Debacker, L. Holsbeek, G. Tapia, S. Gobert, C.R. Joiris, T. Jauniaux, F. Coignoul and J-M. Bouquegneau.

« Diseases of Aquatic Organisms », *in press* **Annexe 1**

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

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Abstract

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

Key words

Heavy metals - PCBs - Cachexia - Guillemots - Belgian coast.

Introduction

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

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

Seabird mortality and, in particular, winter strandings have been carefully reported and monitored along the Belgian coast (Kuyken, 1978) and the neighbouring countries (e.g. Camphuysen and van Franeker, 1992). Camphuysen and Leopold (1994) estimated the number of wintering guillemots in the 130,000 km^2 southern North Sea area at about 235,000 individuals for the 1984 and 1987 October-November peak period. A decline in density occurs around February-March as the birds move back towards the breeding grounds. To what extent birds dying at sea contribute to this decline is unclear, as the percentage of these birds finally reaching the shores is unknown.

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

Material and methods

Collection and storage

A regular and systematic collection of stranded seabirds was organised along the 67 kilometers of the Belgian shore during 6 successive winters (1989-90 to 1994-95). Two hundred fifty one dead guillemots were collected from the beaches, 476 still alive went through rehabilitation centres where they eventually died. Putrified specimens were discarded. Collected carcasses were kept frozen until necropsy was performed at the Pathology Department of the Veterinary College, Liège University, using a consistent protocol (Dorrestein and van der Hage, 1993). They were weighed, oil contamination on plumage and/or in intestinal tract and lesions were noted. Nutritional state, absence of subcutaneous fat and light to severe atrophy of pectoral muscle (visible signs of cachexia) were evaluated on a range from 0 to 3; specifically: 0: presence of subcutaneous fat, normal pectoral muscle; 1: absence of fat and slight pectoral muscle atrophy; 2: moderate pectoral muscle atrophy; 3: severe pectoral muscle atrophy. For statistics, group 0 was tested against groups 1, 2, and 3 to compare normal v/s cachectic birds. Necropsy technique involved opening of bodily cavities, dissection of the digestive tract, examination of the respiratory, urinary and genital systems (Jauniaux *et al.*, 1996). Intestinal serosal surface congestion, hyperaemic and thickened intestinal wall, and hemorrhagic content were used as parameters for acute and hemorrhagic gastro-enteropathy diagnosis (Dorrestein and van der Hage, 1996). Parasites were identified on 248 guillemots and have been previously reported by Brosens *et al.* 1996. Respiratory tract mycetes (*Aspergillus spp.*) have been identified on 7 guillemots out of 198 (Jauniaux and Coignoul, 1994). For bacteriology, all 727 birds have been evaluated for evidence of intestinal salmonellosis following a classic isolation procedure reported elsewhere (Jauniaux *et al.*, 1996). Three birds were positive for *Salmonella* (two cases of *S. enteritidis* and one case of undetermined *Salmonella spp.*). Histopathology was restricted to lesions observed at necropsy. Most lesions were histolytic and bore freezing artifacts. The only significant lesions seen in guillemots were in relation with infectious agents such as *Aspergillus spp.* (see above). No test was used for virus isolation. Two age classes were considered based on the presence of cloacal *bursa fabricii* (Camphuysen and van Franeker, 1992): class I comprising juvenile (1st winter) and immature (2nd and 3rd winter) birds; class II (4th winter and on) consisting of mature, but not necessarily breeding birds. From a total of 727 birds, 339 (170 beached dead, 169 rehabilitation centres) were dissected and samples of liver, kidney and pectoral muscle were collected for analysis of total Hg, organic Hg, polar lipids and PCBs (Laboratory for Ecotoxicology and Polar Ecology of Brussels Free University) and heavy metals, metallothioneins and total lipid (Oceanology Department of Liège University).

Total mercury and organic mercury analyses

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

Organic mercury (MeHg) concentrations were measured by ECD semi-capillary gas chromatography on a Packard 437 following a toluene (Merck 8389) three step extraction

(Uthe *et al.*, 1972). Fresh weight/dry weight ratio was determined by lyophilising. Mercury concentrations were expressed as µg/g dry weight.

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

Other trace element analysis

Atomic absorption spectrophotometry (ARL 3510) was used to determine heavy metal concentrations (Cu, Zn, Cd, Fe). Pb, Ni, Cr and Ti contents were also determined but the results most often were below the detection limits and will not be discussed. After being weighed and dried during 48 hours at 110°C, samples were digested with a mixed solution of chloric (Merck 317) and nitric (Merck 456) acids (1:3,v:v) and slowly heated to 100°C until complete digestion. The samples were then diluted, filtered and analysed. Parallel to the samples, a set of certified material samples (CRM 278 Community Bureau of Reference, Commission of the European Communities) was also analysed to ensure the method's sensitivity. Recoveries ranged from 92 to 102 % for Cu, Zn and Fe and 80 % for Cd. Limits of detection were 0.01µg/g dw for Cu, 0.33 for Zn and 0.22 for Cd. Concentrations are expressed as µg/g dry weight.

PCB analysis

PCB residues were determined by ECD-gas chromatography on a Shimadzu GC14A using a 30 m fused silica CPSil 8CB capillary column following an hexane extraction (Jansen

26.836.64) and florisil (Macherey-Nagel 81571) clean-up. PCBs were identified using a congener mixture including IUPAC congeners 28, 31, 52, 101, 118, 138, 153, 156, 170, 180 and 194. Results were expressed as µg/g dry weight. Since the sample PCB patterns did not sufficiently coincide with Aroclor 1254 or 1260 patterns, results were expressed as ΣPCB, or the sum of the 10 individually identified congeners, which represent ±35% of the total PCB load.

Sample preparation and lipids analysis

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

Statistical analysis

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

Results and discussion

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

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

igure 1

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

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

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

igure 2

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

igure 3 a and b

Acute hemorrhagic gastro-enteropathy showed no clear relation with levels of stable contaminants, except in case of organic Hg which was found in higher concentrations in the kidney of animals which had developed acute hemorrhagic gastro-enteropathy (Table 3 b). The inflammatory nature of the intestinal lesion could not be conclusively assessed, due to the poor quality of the material for histopathology. However, we felt the lesion was worth mentioning, since it affected 61 % of the birds and had no clear correlation with decay. Previous reports mentioned a hemorrhagic gastro-enteritis as a terminal lesion, stress related, in marine birds

(Dorrestein and van der Hage, 1993; Leighton, 1993). In addition, parasitological and bacteriological examinations failed to isolate a likely infectious cause for that lesion (Jauniaux and Coignoul, 1994; Jauniaux *et al.*, 1996; Brosens *et al.*, 1996). No significant overall trend could be linked to the status of oiling when comparing non-oiled and externally oiled birds, which could partially be explained by the fact that external oiling may have occured as a postmortem artifact. However, significant differences in metal content appeared at different levels when comparing non-oiled guillemots with individuals which were oiled both externally and internally; it is yet unclear whether or not these differences can be linked to changes in the metabolisation of the involved metals in response to oiling.

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

Conclusions

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

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Legends

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

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

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

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

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

Table 4: Comparison of trace element concentrations (µg/g dw), expressed as a mean ± standard deviation, in *Uria aalge* of different origins, nd = non determined, <dl = below detection limit.

Figure 1: Overall stranding (percentage of total number) of guillemots (this work) compared to their densities (number/km^2) in the southern North Sea (Camphuysen and Leopold, 1994).

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

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

« **Diseases of Aquatic Organisms** », *in press*

Annexe 1

Table 1

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

« Diseases of Aquatic Organisms », *in press*

Annexe 1

Table 2

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

	Liver			*Kidney*			*Muscle*		
	Beach	Centre	p =	Beach	Centre	p =	Beach	Centre	p =
Cu	52 ± 17 52 14 - 100 *(144)*	51 ± 26 50 10 - 152 *(104)*	ns	28 ±12 27 1.1 - 76.3 *(110)*	31 ±13 31 2 - 74 *(53)*	ns	18 ± 6 18 9 - 53 *(145)*	20 ± 11 18 3 - 90 *(107)*	ns
Zn	145 ±39 138 66 - 328 *(144)*	↗ 168 ±46 158 84 - 413 *(104)*	**< 0.01**	169 ± 41 168 41 - 284 *(111)*	173 ± 46 176 37 - 286 *(54)*	ns	60 ± 14 59 31 - 131 *(145)*	↗ 73 ±31 67 36 - 235 *(107)*	**< 0.01**
Fe	2549 ±1354 2274 393 - 5928 *(144)*	↗ 3557 ± 1564 3468 775 - 7946 *(104)*	**< 0.01**	613 ± 294 569 122 - 2376 *(111)*	700 ± 257 668 367 - 1759 *(53)*	< 0.05	669 ± 241 641 337 - 2428 *(145)*	↗ 903 ± 784 732 78 - 5724 *(107)*	**< 0.01**
Cd	2.4 ± 1.6 2.1 <dl - 10.1 *(144)*	2.5 ± 1.9 2.1 <dl - 9.7 *(104)*	ns	7.8 ± 6.5 6.4 <dl - 39.9 *(111)*	6.2 ±5.3 4.7 <dl - 30.2 *(54)*	ns	<dl	<dl	
Total Hg	5.9 ± 2.9 5,4 0.8 - 20.7 *(156)*	7.9 ± 6.3 6,0 1.2 - 35.8 *(125)*	< 0.05	4.6 ± 3.0 4,0 1.0 - 23.8 *(90)*	5.7 ± 2.4 4,5 4.4 - 9.3 *(4)*	ns	2.1 ± 1.1 1,8 0.3 - 6.7 *(163)*	↗ 3.8 ± 3.4 2,8 0.4 - 23.2 *(139)*	**< 0.01**
Org. Hg	4.6 ± 2.2 4,1 0.8 - 14.1 *(138)*	↗ 6.6 ± 5.4 5,0 1.3 - 32.3 *(105)*	**< 0.01**	3.3 ± 1.5 3,0 1.0 - 6.9 *(55)*	5.2 ± 1.8 4,6 3.6 - 7.8 *(4)*	< 0.05	1.6 ± 0.8 1,4 0.3 - 4.9 *(136)*	↗ 3.1 ± 2.8 2,2 0.4 - 17.8 *(114)*	**< 0.01**
Inorg.Hg	1.1 ± 1.1 0,9 0.0 - 6.5 *(135)*	1.3 ± 1.6 0,9 0.0 - 10.8 *(105)*	ns	0.9 ± 0.7 0,7 0.0 - 2.6 *(54)*	0.6 ± 0.7 0,5 0.0 - 1.5 *(4)*	ns	0.4 ± 0.4 0,3 0.0 - 1.9 *(136)*	0.6 ± 0.8 0,4 0.0 - 5.3 *(113)*	ns
Sum PCB	5.7 ± 6.0 3,5 0.3 - 27.2 *(130)*	↗ 11.7 ± 13.0 8,7 1.0 - 60.4 *(68)*	**< 0.01**	3.4 ± 2.8 2,6 0.1 - 12.8 *(88)*	2.6 ± 1.6 2,2 1.1 - 4.7 *(4)*	ns	2.1 ± 1.8 1,6 0.1 - 10.5 *(130)*	↗ 5.4 ± 10.0 3,0 0.2 - 81.9 *(77)*	**< 0.01**
Total lipids	0.18 ± 0.07 0.17 0.03 - 0.60 *(120)*	0.16 ±0.07 0.15 0.06 - 0.33 *(32)*	ns	nd	nd		0.10 ± 0.08 0,08 0.01 - 0.63 *(119)*	↗ 0.14 ± 0.12 0,10 0.05 - 0.65 *(31)*	**< 0.01**
Polar lipids	0.11 ± 0.03 0,11 0.04 - 0.29 *(130)*	0.11 ± 0.04 0,10 0.07 - 0.27 *(68)*	ns	0.12 ± 0.02 0,12 0.03 - 0.17 *(88)*	0.07 ± 0.04 0,08 0.01 - 0.11 *(4)*	< 0.05	0.04 ± 0.03 0,03 0.01 - 0.16 *(130)*	0.05 ± 0.04 0,04 0.01 - 0.19 *(77)*	ns

Table 3 a

	Age		**Sex**		**Cachexia**		**Gastro-enteropathy**		**Oiling**		
	Class I	Class II									
	Juv. + imm.	**Adult**	**Male**	**Female**	-	+	-	+	**no**	**E. + I.**	**E.**
Body mass	707 ± 109 688 *(92)*	761 ± 133 725 *(49)*	725 ± 123 700 *(86)*	717 ± 117 700 *(65)*	781 ± 140 760 *(40)*	708 ± 116 680 *(126)*	748 ± 132 707 *(64)*	711 ± 120 687 *(102)*	698 ± 116 680 *(75)*	745 ± 123 715,0 *(51)*	747 ± 137 700,0 *(41)*
Liver											
Cu	51 ± 17 51 *(75)*	52 ± 17 53 *(42)*	53 ± 17 53 *(74)*	51 ± 16 51 *(54)*	48 ± 20 45 *(36)*	53 ± 16 52 *(108)*	52 ± 17 57 *(55)*	52 ± 17 52 *(89)*	51 ± 16 52 *(66)*	52 ± 16 50 *(39)*	53 ± 21 55 *(38)*
Zn	145 ± 39 137 *(75)*	150 ± 38 147 *(42)*	147 ± 46 134 *(74)*	145 ± 28 143 *(54)*	129 ± 33 125 *(36)*	151 ± 40 143 *(108)*	148 ± 47 130 *(55)*	144 ± 33 140 *(89)*	142 ± 34 136 *(66)*	150 ± 30 145 *(39)*	147 ± 54 132 *(38)*
Fe	2325 ± 1202 2178 *(75)*	2867 ± 1438 2554 *(42)*	2353 ± 1087 2231 *(74)*	2758 ± 1603 2372 *(54)*	2528 ± 1391 2130 *(36)*	2557 ± 1347 2320 *(108)*	2701 ± 1484 2337 *(55)*	2455 ± 1266 2260 *(89)*	2509 ± 1424 2318 *(66)*	2499 ± 1335 2189 *(39)*	2679 ± 1288 2362 *(38)*
Cd	2.2 ± 1.6 1,9 *(75)*	2.7 ± 1.4 2,3 *(42)*	2.4 ± 1.5 2,2 *(74)*	2.7 ± 1.8 2,2 *(54)*	2.5 ± 2.3 2,0 *(36)*	2.4 ± 1.3 2,2 *(108)*	2.7 ± 2.1 2,3 *(55)*	2.2 ± 1.3 2,0 *(89)*	2.7 ± 2.1 2,1 *(66)*	2.2 ± 1.2 2,0 *(39)*	2.2 ± 1.2 2,1 *(38)*
Total Hg	6.4 ± 3.2 5,7 *(87)*	5.1 ± 2.4 4,7 *(45)*	4.3 ± 2.1 4,1 *(20)*	5.9 ± 2.4 6,0 *(24)*	5.3 ± 2.3 4,9 *(37)*	6.1 ± 3.1 5,5 *(118)*	5.8 ± 2.8 5,5 *(57)*	6.0 ± 3.1 5,3 *(98*	5.3 ± 1.9 5,3 *(71)*	6.9 ± 3.9 6,9 *(43)*	5.7 ± 3.0 5,5 *(41)*
Org. Hg	4.9 ± 2.4 4,2 *(75)*	4.2 ± 2.1 3,9 *(41)*	3.5 ± 1.8 3,0 *(17)*	4.9 ± 2.1 4,8 *(23)*	4.3 ± 2.0 4,3 *(34)*	4.7 ± 2.3 4,0 *(103)*	4.6 ± 2.3 4,2 *(51)*	4.6 ± 2.3 3,9 *(86)*	4.2 ± 1.3 4,1 *(63)*	5.1 ± 2.8 4,3 *(37)*	4.6 ± 2.7 3,9 *(37)*
Inorg. Hg	1.2 ± 1.3 0,9 *(74)*	1.0 ± 1.1 0,7 *(40)*	1.0 ± 1.0 0,7 *(17)*	1.1 ± 1.2 0,5 *(22)*	0.8 ± 0.9 0,6 *(33)*	1.2 ± 1.2 0,9 *(101)*	1.1 ± 1.0 0,8 *(49)*	1.2 ± 1.2 0,9 *(85)*	1.0 ± 0.8 0,9 *(63)*	1.4 ± 1.5 0,8 *(34)*	1.2 ± 1.2 0,7 *(37)*
Sum PCB	5.2 ± 5.8 3,3 *(68)*	7.5 ± 6.8 5,5 *(40)*	4.2 ± 2.6 4,1 *(17)*	10.4 ± 7.8 9,8 *(22)*	2.8 ± 2.9 1,7 *(31)*	6.6 ± 6.4 4,2 *(99)*	6.0 ± 6.4 3.5 *(46)*	5.6 ± 5.8 3,5 *(84)*	4.8 ± 4.8 3,3 *(68)*	8.1 ± 7.1 5,0 *(22)*	6.0 ± 6.9 3,5 *(39)*
Total lipids	0.17 ± 0.08 0,17 *(58)*	0.18 ± 0.05 0,18 *(35)*	0.17 ± 0.08 0,17 *(60)*	0.18 ± 0.05 0,18 *(44)*	0.18 ± 0.07 0,18 *(32)*	0.18 ± 0.07 0,17 *(88)*	0.19 ± 0.05 0,18 *(43)*	0.17 ± 0.07 0,17 *(77)*	0.18 ± 0.07 0,17 *(66)*	0.18 ± 0.06 0,19 *(17)*	0.18 ± 0.06 0,17 *(36)*
Polar lipids	0.11 ± 0.03 0,11 *(68)*	0.11 ± 0.04 0,10 *(40)*	0.12 ± 0.04 0,12 *(17)*	0.10 ± 0.02 0,1 *(22)*	0.11 ± 0.05 0,1 *(31)*	0.11 ± 0.03 0,11 *(99)*	0.11 ± 0.03 0,10 *(46)*	0.11 ± 0.03 0,11 *(84)*	0.11 ± 0.03 0,11 *(68)*	0.09 ± 0.02 0,09 *(22)*	0.12 ± 0.04 0,11 *(39)*

Table 3 b

	Age		**Sex**		**Cachexia**		**Gastro-enteropathy**		**Oiling**		
	Class I	Class II									
	Juv. + imm.	**Adult**	**Male**	**Female**	-	+	-	+	**no**	**E. + I.**	**E.**
Kidney											
Cu	28 ± 14 27 *(53)*	29 ± 11 29 *(33)*	28 ± 13 27 *(56)*	29 ± 13 29 *(40)*	22 ± 12 18 *(27)*	↗ 30 ± 12 29 *(83)*	27 ± 13 27 *(38)*	28 ± 12 27 *(72)*	29 ± 11 28 *(61)*	25 ± 13 25 *(12)*	27 ± 14 27 *(36)*
Zn	170 ± 42 170 *(53)*	175 ± 40 182 *(33)*	169 ± 42 164 *(56)*	177 ± 39 187 *(40)*	150 ± 46 147 *(28)*	↗ 176 ± 37 177 *(83)*	161 ± 45 160 *(37)*	174 ± 38 173 *(73)*	174 ± 41 176 *(61)*	159 ± 35 155 *(12)*	164 ± 42 167 *(37)*
Fe	597 ± 320 563 *(53)*	622 ± 259 629 *(33)*	610 ± 216 594 *(56)*	592 ± 358 559 *(40)*	748 ± 278 694 *(28)*	↘ 567 ± 287 540 *(83)*	617 ± 270 604 *(37)*	611 ± 307 563 *(73)*	542 ± 225 529 *(61)*	688 ± 259 689 *(12)*	702 ± 375 650 *(37)*
Cd	5.9 ± 4.0 4,9 *(53)*	↗ 10.4 ± 6.8 9,2 *(33)*	7.2 ± 6.2 6,1 *(56)*	9.4 ± 7.3 7,1 *(40)*	9.2 ± 10.0 5,9 *(28)*	7.3 ± 4.9 6,5 *(83)*	8.6 ± 8.1 6,5 *(38)*	7.3 ± 5.6 6,3 *(73)*	8.3 ± 7.5 6,3 *(61)*	4.4 ± 2.5 3,8 *(12)*	7.9 ± 5.6 6,8 *(37)*
Total Hg	4.9 ± 3.5 4,0 *(45)*	4.3 ± 2.6 3,5 *(24)*	3.9 ± 2.1 3,6 *(12)*	5.1 ± 3.0 3,8 *(11)*	4.0 ± 1.9 3,5 *(26)*	4.8 ± 3.2 4,1 *(64)*	4.0 ± 2.2 3,5 *(32)*	4.9 ± 3.2 4,2 *(58)*	4.0 ± 1.5 4,0 *(49)*	↗ 8.7 ± 6.9 7,2 *(7)*	4.5 ± 2.6 3,5 *(33)*
Org. Hg	3.6 ± 1.5 3,1 *(25)*	2.9 ± 1.6 2,5 *(16)*	2.9 ± 1.3 2,5 *(9)*	3.1 ± 2.0 2,5 *(6)*	2.9 ± 1.5 2,4 *(19)*	3.5 ± 1.4 3,3 *(36)*	2.6 ± 0.9 2,4 (18)	↗ 3.6 ± 1.6 3,7 (37)	3.0 ± 1.0 3,0 *(33)*	4.9 ± 1.7 5,9 *(3)*	3.5 ± 1.8 3,0 *(18)*
Inorg. Hg	0.8 ± 0.7 0,7 *(25)*	0.9 ± 0.8 0,6 *(15)*	0.8 ± 0.9 0,5 *(8)*	1.1 ± 0.7 1,0 *(6)*	0.8 ± 0.6 0,7 *(18)*	0.9 ± 0.7 0,8 *(36)*	0.8 ± 0.6 0,8 *(18)*	0.9 ± 0.7 0,7 *(36)*	0.8 ± 0.6 0,8 *(32)*	1.2 ± 0.8 0,9 *(3)*	0.9 ± 0.8 0,7 *(18)*
Sum PCB	3.0 ± 2.4 2,4 *(43)*	3.3 ± 3.1 2,8 *(25*	2.6 ± 1.9 2,8 *(13)*	4.5 ± 3.9 3,4 *(11)*	2.9 ± 2.8 2,1 *(27)*	3.6 ± 2.8 2,8 *(61)*	3.6 ± 3.1 2,9 *(32)*	3.3 ± 2.7 2,6 *(56)*	3.2 ± 2.7 2,5 *(46)*	4.9 ± 4.4 2,8 *(7)*	3.2 ± 2.7 2,8 *(34)*
Total lipids	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Polar lipids	0.12 ± 0.02 0,12 *(43)*	0.12 ± 0.03 0,13 *(25)*	0.12 ± 0.03 0,13 *(13)*	0.11 ± 0.03 0,11 *(11)*	0.11 ± 0.02 0,11 *(27)*	0.12 ± 0.02 0,12 *(61)*	0.11 ± 0.02 0,12 *(32)*	0.12 ± 0.02 0,12 *(56)*	0.12 ± 0.2 0,12 *(46)*	0.11 ± 0.03 0,11 *(7)*	0.12 ± 0.02 0,12 *(34)*

Table 3 c

	Age		Sex		Cachexia		Gastro-enteropathy		Oiling		
	Class I	Class II									
	Juv. + imm.	**Adult**	**Male**	**Female**	-	+	-	+	no	E. + I.	E.
Muscle											
Cu	18 ± 6 18 *(77)*	16 ± 4 16 *(41)*	18 ± 6 18 *(76)*	18 ± 5 17 *(53)*	18 ± 5 18 *(37)*	18 ± 6 18 *(108)*	17 ± 5 17 *(56)*	19 ± 6 18 *(89)*	20 ± 7 19 *(65)*	16 ± 4 16 *(41)*	18 ± 5 18 *(38)*
Zn	61 ± 15 59 *(77)*	58 ± 11 57 *(41)*	58 ± 16 55 *(76)*	61 ± 11 61 *(53)*	53 ± 12 52 *(37)*	62 ± 14 62 *(108)*	57 ± 13 55 *(56)*	62 ± 15 61 *(89)*	63 ± 15 63 *(65)*	56 ± 11 54 *(41)*	59 ± 15 55 *(38)*
Fe	693 ± 292 643 *(77)*	612 ± 130 584 *(41)*	688 ± 297 641 *(76)*	649 ± 131 640 *(53)*	586 ± 145 580 *(37)*	697 ± 260 645 *(108)*	646 ± 238 599 *(56)*	683 ± 242 663 *(89)*	711 ± 264 663 *(65)*	601 ± 107 586 *(41)*	674 ± 289 648 *(38)*
Cd	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
Total Hg	2.2 ± 1.1 1,9 *(89)*	2.0 ± 1.2 1,7 *(49)*	1.6 ± 0.9 1,4 *(20)*	2.3 ± 1.4 1,9 *(28)*	2.0 ± 0.8 2,1 *(39)*	2.1 ± 1.2 1,7 *(122)*	2.2 ± 1.3 2,0 *(62)*	2.0 ± 1.0 1,7 *(99)*	1.9 ± 0.8 1,8 *(72)*	2.5 ± 1.3 2,3 *(49)*	2.0 ± 1.3 1,5 *(41)*
Org. Hg	1.7 ± 0.8 1,6 *(77)*	1.5 ± 0.9 1,3 *(39)*	1.2 ± 0.8 1,0 *(15)*	1.7 ± 0.9 1,4 *(23)*	1.5 ± 0.7 1,5 *(33)*	1.7 ± 0.9 1,4 *(101)*	1.7 ± 1.0 1,4 *(49)*	1.6 ± 0.8 1,4 *(85)*	1.5 ± 0.6 1,4 *(64)*	1.8 ± 0.9 1,5 *(35)*	1.6 ± 1.1 1,3 *(36)*
Inorg. Hg	0.4 ± 0.4 0,3 *(77)*	0.4 ± 0.4 0,2 *(39)*	0.3 ± 0.3 0,2 *(15)*	0.4 ± 0.5 0,3 *(23)*	0.4 ± 0.4 0,3 *(33)*	0.4 ± 0.4 0,3 *(101)*	0.4 ± 0.4 0,3 *(49)*	0.4 ± 0.4 0,3 *(85)*	0.3 ± 0.4 0,2 *(64)*	0.6 ± 0.4 0,5 *(35)*	0.3 ± 0.4 0,2 *(36)*
Sum PCB	1.8 ± 1.4 1,3 *(68)*	2.6 ± 2.1 2,1 *(40)*	1.9 ± 0.9 1,6 *(17)*	3.1 ± 2.5 2,5 *(22)*	2.5 ± 2.3 2,0 *(31)*	2.0 ± 1.5 1,6 *(99)*	2.4 ± 1.9 1,9 *(46)*	2.0 ± 1.7 1,6 *(84)*	1.7 ± 1.4 1,3 *(68)*	2.9 ± 2.5 2,0 *(22)*	2.4 ± 1.7 2,0 *(39)*
Total lipids	0.08 ± 0.04 0,08 *(59)*	0.11 ± 0.08 0,08 *(33)*	0.11 ± 0.09 0,08 *(61)*	0.09 ± 0.05 0,08 *(42)*	0.13 ± 0.11 0,11 *(32)*	0.09 ± 0.05 0,08 *(87)*	0.12 ± 0.11 0,08 *(43)*	0.09 ± 0.04 0,08 *(76)*	0.09 ± 0.05 0,08 *(65)*	0.09 ± 0.05 0,07 *(17)*	0.13 ± 0.11 0,10 *(36)*
Polar lipids	0.04 ± 0.02 0,03 *(68)*	0.05 ± 0.04 0,04 *(40)*	0.06 ± 0.033 0,04 *(17)*	0.04 ± 0.03 0,03 *(22)*	0.07 ± 0.04 0,05 *(31)*	0.04 ± 0.01 0,03 *(99)*	0.05 ± 0.04 0,04 *(46)*	0.04 ± 0.02 0,03 *(84)*	0.03 ± 0.01 0,03 *(68)*	0.05 ± 0.03 0,04 *(22)*	0.05 ± 0.04 0,04 *(39)*

« Diseases of Aquatic Organisms », *in press*

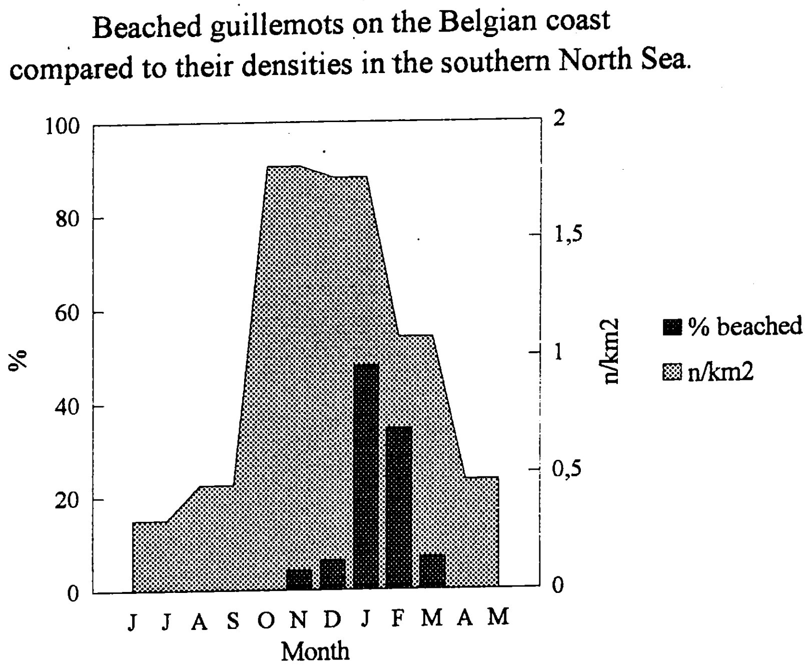
Annexe 1

Table 4

		Time	Place	Cu	Zn	Cd	Total Hg	
Liver	n = 51	1970-1981	Belgian coast	nd	nd	nd	7.2 ± 2.4	Delbeke et al., 1984
	n = 83	April to Nov. 1988	Northwest Scotland	range 12.9 - 16.1	range 58.4 - 69.7	range 1.4 - 2.5	range 0.9 - 3.7	Stewart et al., 1994
	n = 10	summer 1992 and 1993	Hornoya North. Norway	20.0 ± 2.9	86.7 ± 14.9	3.1 ± 1.1	1.9 ± 0.4	Wenzel and Gabrielsen, 1994
	n = 143	***winter 1990 to 95***	***Belgian coast***	***52 ± 17***	***145 ± 39***	***2.4 ± 1.6***	***6.1 ± 3.4***	***this study***
Kidney	n = 9	1970-1981	Belgian coast	nd	nd	nd	4.4 ± 1.7	Delbeke et al., 1984
	n = 10	summer 1992 and 1993	Hornoya North. Norway	14.4 ± 1.9	114 ± 13	24.1 ± 7.5	1.5 ± 0.2	Wenzel and Gabrielsen, 1994
	n = 83	April to Nov. 1988	Northwest Scotland	range 12.3 - 15.2	range 59.3 - 74.1	range 1.6 - 11.7	range 0.8 - 3.9	Stewart et al., 1994
	n = 143	***winter 1990 to 95***	***Belgian coast***	***28 ± 12***	***169 ± 41***	***7.8 ± 6.6***	***4.6 ± 2.9***	***this study***
Muscle	n = 24	April to Nov. 1988	Northwest Scotland	range 10.2 - 14.0	range 20.9 - 26.0	nd	range 0.5 - 1.8	Stewart et al., 1994
	n = 10	summer 1992 and 1993	Hornoya North. Norway	19.2 ±0.9	49.3 ± 3.3	0.2 ± 0.1	0.4 ± 0.1	Wenzel and Gabrielsen, 1994
	n = 143	***winter 1990 to 95***	***Belgian coast***	***18 ± 6***	***60 ± 14***	***<dl***	***2.1 ± 1.2***	***this study***

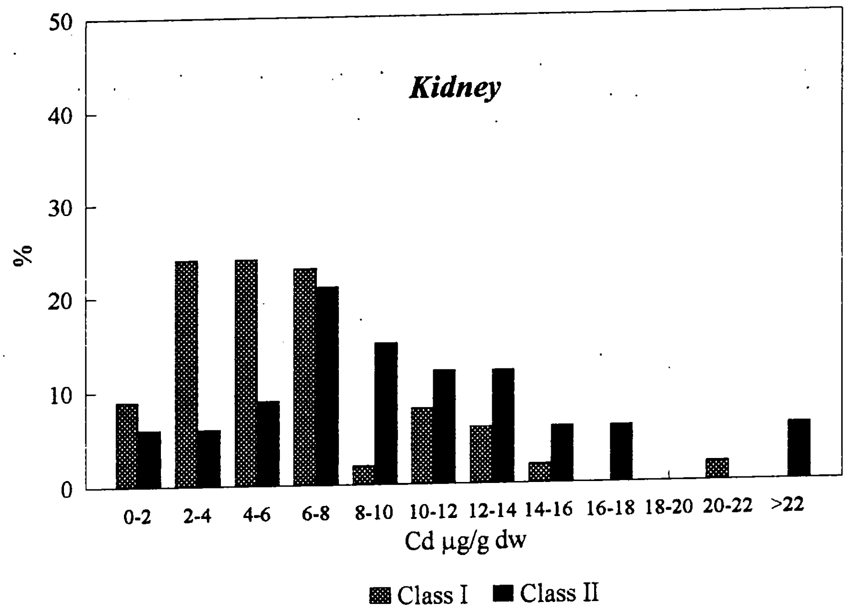
« Diseases of Aquatic Organisms », *in press* **Annexe 1**

Fig. 1



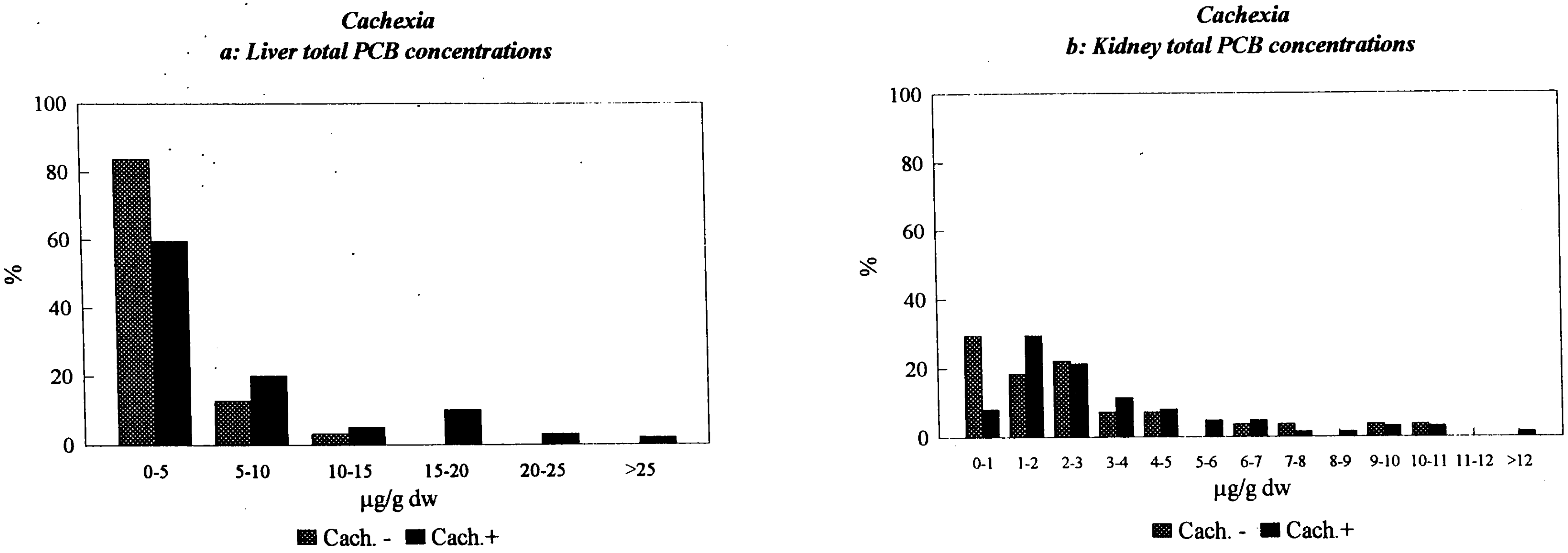
Beached guillemots on the Belgian coast compared to their densities in the southern North Sea.

Fig. 2



Kidney
%
50
40
30
20
10
0
0-2
2-4
4-6
6-8
8-10
10-12
12-14
14-16
16-18
18-20
20-22
>22
Cd µg/g dw
▨ Class I
■ Class II

Fig. 3 a and b


Cachexia
a: Liver total PCB concentrations
%
100
80
60
40
20
0
0-5
5-10
10-15
15-20
20-25
>25
µg/g dw
▨ Cach. - ■ Cach.+
Cachexia
b: Kidney total PCB concentrations
%
100
80
60
40
20
0
0-1
1-2
2-3
3-4
4-5
5-6
6-7
7-8
8-9
9-10
10-11
11-12
>12
µg/g dw
▨ Cach. - ■ Cach.+

Comparative Biochemistry and Physiology, 113 C, N°2, pp 135-139, 1996.

Annexe 2

Role of metallothioneins in metal regulation by the guillemot *Uria aalge*.

J.M. Bouquegneau, V. Debacker, S. Gobert and S. Havelange.

Annexe 2

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Role of Metallothioneins in Metal Regulation by the Guillemot *Uria aalge*

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ABSTRACT. Guillemots, like other seabird species living in the North Sea, appear to be heavily contaminated by copper. Metallothioneins are present in both liver and kidney but, at least in the specimens stranded along the Belgian coast, fail to maintain constant the copper, zinc and cadmium load of the high molecular weight soluble proteins of both organs, stressing the potential toxic role of these metals, mainly copper. COMP BIOCHEM PHYSIOL 113C, 135–139, 1996.

KEY WORDS. Metallothioneins, copper, cadmium, zinc, heavy metals regulation, seabirds, *Uria aalge*, Belgian coast

INTRODUCTION

In a previous paper (4) we reported that the mortality of nonoiled guillemots *Uria aalge* stranded along the Belgian coast during the winter 1992–1993 was attributed to the development of hemorrhagic gastroenteritis which was related, via cachexia, to high levels of copper, zinc and cadmium. No data are available about copper, zinc and cadmium levels of the species, but the copper load of their livers appeared to be very high. Indeed, copper concentrations in other seabird livers are known to exhibit low variation on both interspecies and geographical bases and mean concentrations tend to be around 6 μg/gFW with maximal values of 10 μg/gFW (from data collected in Antarctica, in Australia, in U.S., New Zealand and Spitzbergen (17)). Data for Atlantic Canadian seabirds are within the same range (8). However, in the North Sea, the mean copper content of seabirds' livers is significantly higher: 13.5 μg/gFW in the guillemot *Uria aalge*, 9.1 μg/gFW in the kittiwake *Rissa tridactyla*, 23.0 μg/gFW in the black headed gull *Larus ridibundus*, and 16.8 μg/gFW in the common scoter *Melanitta nigra* (2), with maximal values of 29.2, 14.9, 113.3 and 43.8 μg/gFW respectively (1). Zinc and cadmium content of the guillemots' livers were on the contrary within the range reported in other seabird species.

In man, it is known that ingested copper salts are able to induce gastroenteritis and that zinc salts are irritative for the digestive tract (11). On another hand, cadmium induces cachexia and enteropathy in the Japanese Quail (9,15). So we consider that copper, zinc and cadmium, providing their excess is not stored in the tissues under a detoxified form, may have a potential harmful effect on the guillemots stranded along the Belgian coast.

In order to assess the potential toxicity of these metals, we report hereafter their speciation, with special reference to metallothioneins, in the liver and kidney of the guillemots stranded along the Belgian coast.

MATERIALS AND METHODS

Guillemots

The guillemots were collected during winters 1992–1993 and 1993–1994 along the Belgian coast (North Sea) or in the rehabilitation centers of Zwin, Nieuwpoort, Oostende and Blankenberghe. They were first necropsied and deep-frozen in the Pathology Department of the Veterinary College (University of Liège, Professor F. Coignoul) and then brought to our laboratory. From the animals collected during winter 1992–1993 and during winter 1993–1994, 16 and 13 animals have been studied respectively. To check the validity of the results related to the speciation of heavy metals in tissues collected several hours after the death of the animals, three alive specimens, stranded during the winter 1994–1995, were sacrificed and treated at once.

Preparation of Samples for Metallothionein Determination and Heavy Metal Analysis

Two gFW liver or kidney were homogenized by means of an Ultra-Turrax 18/10 in two volumes of 0.01 Mol ammonium formiate (pH 7.4; Carlo Erba 419735) containing 10 mMol dithiothreitol and then centrifuged (centrifuge ALC 972 R) at 26000 g for 2 hr. The supernatant was filtered on a LKB

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Based on a presentation given at the 15th Annual Conference of the European Society for Comparative Physiology and Biochemistry held in Genoa, Italy, in September 1994. The Conference title was: *Biochemical and physiological effects of pollution and toxicological assessment of environmental quality.*

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Ultrogel AcA 54 column at 4°C with the same buffer. In these conditions, seabirds metallothioneins were found to be eluted at a volume Ve/Vo comprised between 2 and 2.8 (2,7).

Copper, zinc and cadmium were analysed in the chromatographic fractions, in the supernatant and in the pellet by atomic absorption spectrophotometry (Perkin-Elmer, Model 370A) as described by Noël-Lambot *et al* (12).

RESULTS

The relative quantity of Cu, Zn and Cd bound to metallothioneins (MT), to other cytosolic substances (NMTC, mainly high molecular weight proteins) and to insoluble parts of the tissues (Pellet) in relation to the total load of the metal is reported in figs 1 to 5. No cadmium was found in detectable amount in the chromatographic fractions of the liver of the guillemots.

Cytosolic metallothioneins, high molecular weight soluble proteins and insoluble parts of the liver respectively bind 56, 27 and 17% of the excess copper (Fig. 1). They bind 37, 39 and 24% (Fig. 2), 27, 36 and 37% (Fig. 3) and 22, 49 and 28% (Fig. 4) of the excess renal copper, hepatic and renal zinc respectively. Renal cadmium on the contrary is mainly bound to metallothioneins and to the pellet, except at very high concentrations where a large amount of cadmium is bound to high molecular weight soluble proteins (Fig. 5).

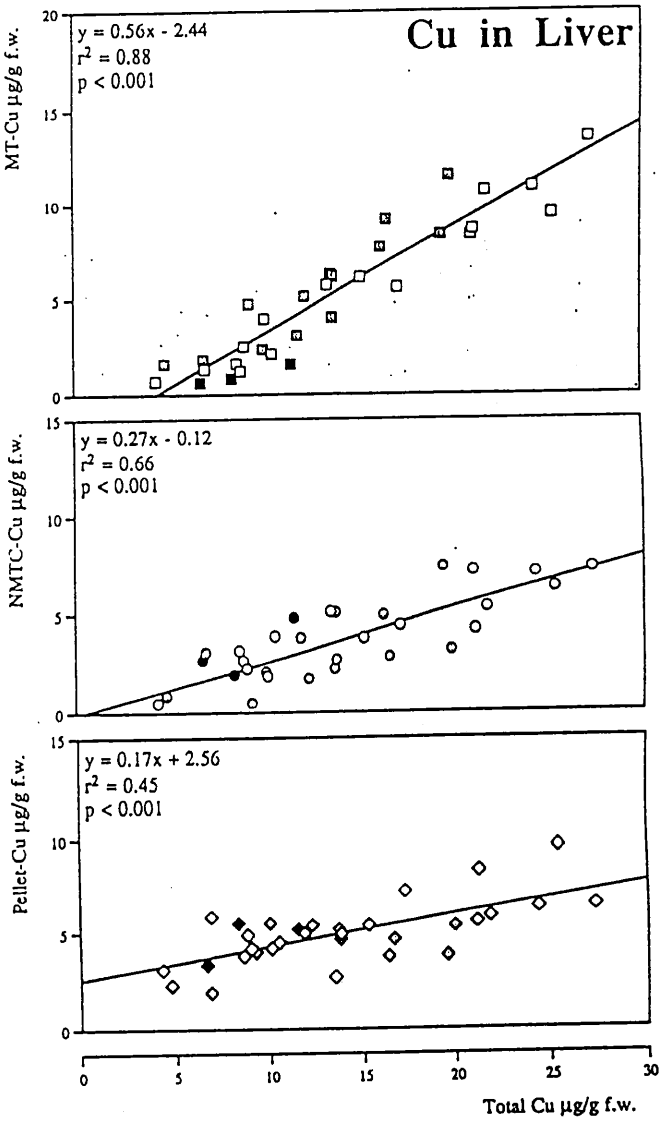

FIG. 1. The relationship of the concentration of copper bound to hepatic metallothioneins (MT-Cu, above), to other cytosolic fractions (NMTC-Cu, middle) and to the insoluble parts (pellet-Cu, below) with the total liver concentration of the metal. White, grey and black points correspond to animals stranded during the 1992–1993, 1993–1994 and 1994–1995 winters, respectively.

DISCUSSION

A major function of metallothionein is commonly regarded as homeostatic regulation of intracellular metals (10,19): binding of copper, zinc and cadmium to the thiol ligands of metallothioneins would prevent the participation of these ions in oxidative attack on membranes, nucleic acids and enzymes. The induction of metallothionein synthesis is known to be associated with an increase of the metal resistance of animals (see *e.g.* 3 and 16 for a review in aquatic animals).

The widespread distribution of metallothioneins in animals is now firmly established and, when considering seabirds, metallothioneins or metallothionein-like proteins have been shown to occur in the kidney and liver of several species: Northern fulmar *Fulmarus glacialis* (13), Atlantic puffin *Fratercula arctica*, herring gull *Larus argentatus*, Leach's storm petrel *Oceanodroma leucorhoa* and double-crested cormorant *Phalacrocorax auritus* (8), black headed gull *Larus ridibundus* (2), guillemot *Uria aalge*, kittiwake *Rissa tridactyla* and common scoter *Melanitta nigra* (1).

In flamingos, Cosson (6) has shown a close correlation between metallothionein concentrations and hepatic copper-zinc or renal copper-zinc and cadmium levels. In egrets, a similar correlation between zinc and metallothioneins was found. This, according to Cosson (6), suggests that zinc is the most important metal influencing metallothionein production. Similarly, Elliot *et al* (8) have found a close relationship between renal Cd and metallothionein concentrations in free-living seabirds (double-crested cormorant, herring gull, Atlantic puffin and storm-petrel) and between renal Hg and metallothionein concentrations in Atlantic puffins.

In the guillemots studied in this paper, data related to dead and alive animals fit very well. The metallothionein content in both liver and kidney increases in heavier contaminated animals (Figs. 1 to 5). Copper is generally known to be a poor inducer of metallothionein (14). However, in the guillemots, most of, but by far not all, the excess of copper is bound to cytosolic metallothionein, probably as a result of the great affinity of the metal for metallothionein compared to cadmium and zinc. However, only a small part of zinc is bound to

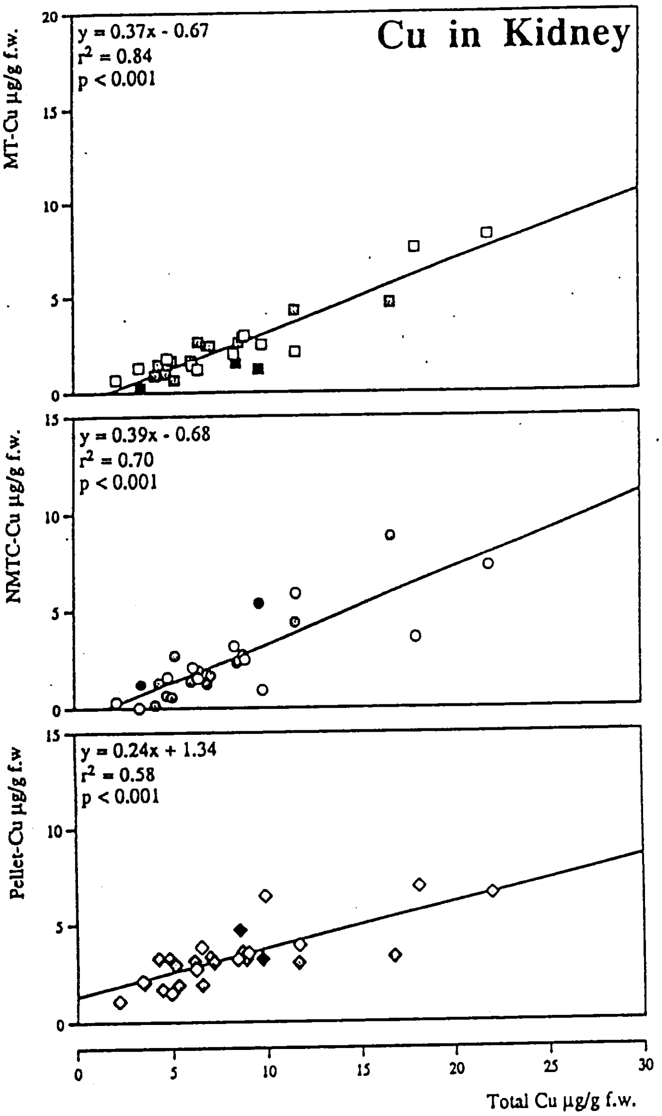

FIG. 2. The relationship of the concentration of copper bound to renal metallothioneins (MT-Cu, above), to other cytosolic fractions (NMTC-Cu, middle) and to the insoluble parts (pellet-Cu, below) with the total kidney concentration of the metal. White, grey and black points correspond to animals stranded during the 1992–1993, 1993–1994 and 1994–1995 winters, respectively.

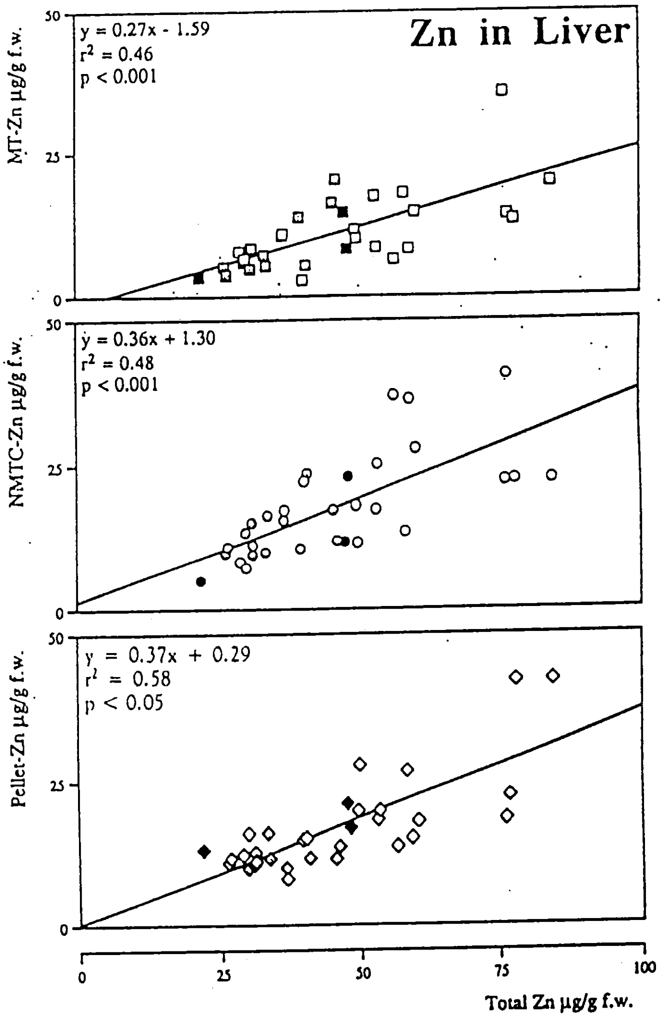

FIG. 3. The relationship of the concentration of zinc bound to hepatic metallothioneins (MT-Zn, above), to other cytosolic fractions (NMTC-Zn, middle) and to the insoluble parts (pellet-Zn, below) with the total liver concentration of the metal. White, grey and black points correspond to animals stranded during the 1992–1993, 1993–1994 and 1994–1995 winters, respectively.

metallothioneins (Figs. 3 and 4). Most of the renal cadmium is bound to metallothioneins, except in highly contaminated animals. This is at variance, for example, with the heavily cadmium polluted limpets of the Bristol Channel whose metallothioneins bind almost all the excess cadmium present in the cytosol (12), and suggests a possible harmful effect of the three metals in the guillemots.

When considering the insoluble parts of the tissues, it is quite difficult to assess the potential toxicity of copper, zinc and cadmium since it is well known that metallothioneins not only occur in the cytosol but also in the lysosomes and other organelles where they polymerize to form insoluble aggregates (5). So, the increase of metals bound to the insoluble part of the tissues could be partly or totally attributed to a binding under detoxified form in those organelles.

On the contrary, the large increase of copper, zinc and cadmium bound to the cytosolic high molecular weight proteins constitutes a important potential threat to the guillemots. A displacement of another metal from the active site of an enzyme or a binding to a deactivating site are the two main molecular mechanisms for metal toxicity which could result from the impairment of the homeostatic function of metallothioneins (18).

It is worth noting that our observations are quite similar if we consider the population stranded during both last winters,

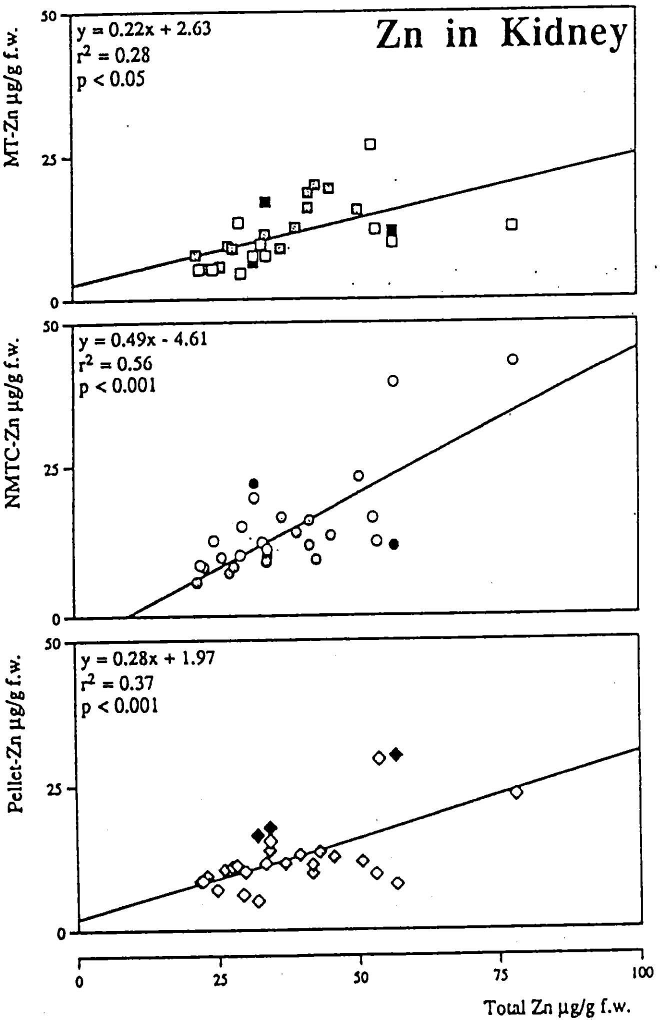

FIG. 4. The relationship of the concentration of zinc bound to renal metallothioneins (MT-Zn, above), to other cytosolic fractions (NMTC-Zn, middle) and to the insoluble parts (pellet-Zn, below) with the total kidney concentration of the metal. White, grey and black points correspond to animals stranded during the 1992–1993, 1993–1994 and 1994–1995 winters, respectively.

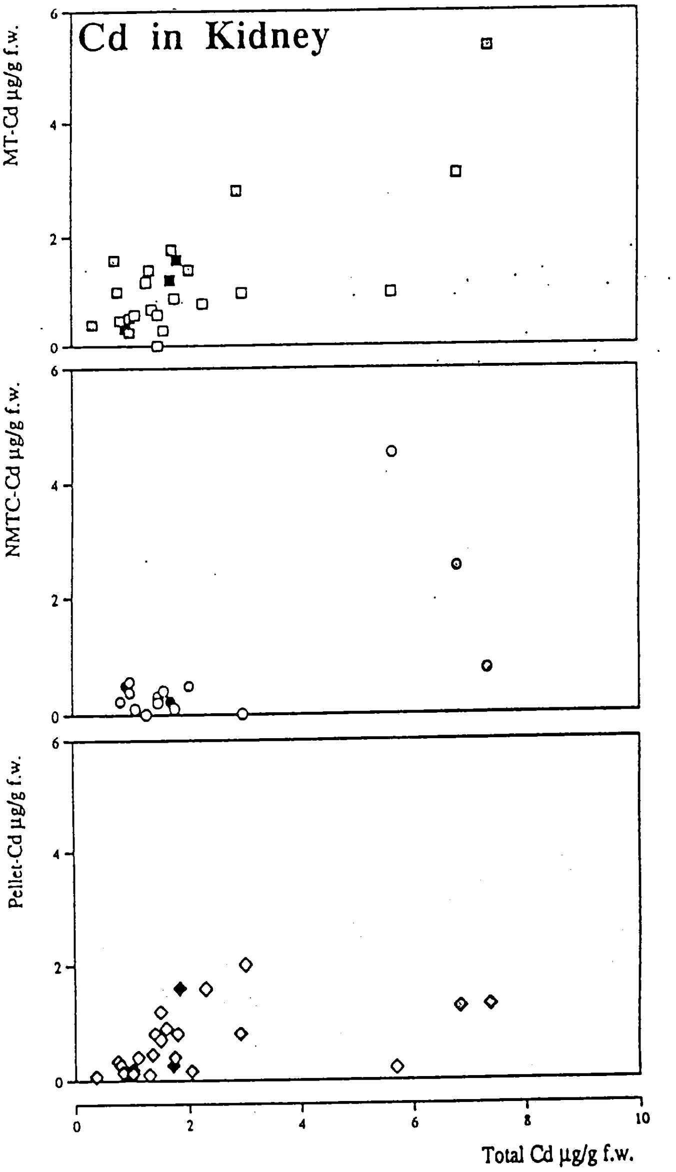

FIG. 5. The relationship of the concentration of cadmium bound to renal metallothioneins (MT-Cd, above), to other cytosolic fractions (NMTC-Cd, middle) and to the insoluble parts (pellet-Cd, below) with the total kidney concentration of the metal. White, grey and black points correspond to animals stranded during the 1992–1993, 1993–1994 and 1994–1995 winters, respectively.

and we conclude that guillemots from the North sea contain high levels of heavy metals, mainly copper, which are likely to induce physiological misfunctioning in the population, leading to cachexia.

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3. Les mammifères marins

3.1. Répartition des espèces.

Sur l'ensemble des hivers considérés, un total de 16 mammifères marins appartenant à 7 espèces ont été analysés.

Espèces	Effectifs (n =)
Phocoena phocoena (marsouin commun)	3
Lagenorhynchus acutus (lagénorhynque à flancs blancs)	1
Lagnorhynchus albirostris (lagénorhynque à bec blanc)	3
Globicephala melaena (globicéphale noir)	1
Physeter macrocephalus (cachalot)	7
Phoca vitulina (phoque commun)	1
TOTAL	**16**

Parmi les différentes espèces de petits cétacés répertoriées en Mer du Nord, certaines sont considérées comme résidentes, notamment les Lagénorhynques à bec blanc et à flancs blancs, le grand dauphin (*Tursiops truncatus*) et le Marsouin commun. Les problèmes principaux auxquels sont confontrées ces populations en Mer du Nord sont directement liés à la détérioration de la qualité de leur environnement et à la dégradation de leur habitat (pollution -métaux lourds, organochlorés, dérivés pétroliers-, sur-exploitation des stocks de pêche, nuisances sonores, captures accidentelles dans les filets de pêche). Les effets indirects (immuno-suppression, diminution de la reproduction...) de la pollution par les métaux lourds et autres toxiques (organochlorés), à long terme, interviennent dans un schèma global de débilitation des individus, conduisant à la mort de ceux-ci (Olsen & Bjørge, 1996; Reijnders & Lankester, 1990).

Le peu d'individus analysés, pour chaque espèce considérée, limite la discusion des résultats, néanmoins ceux-ci conservent leur importance dans le cadre d'un suivi des populations en mer du Nord, notamment grâce à la banque de données internationale établie par l'ICES (International Council for the Exploration of the Sea).

3.2. Etude détaillée: échouage de quatre cachalots (*Physeter macrocephalus*), Koksijde, 1994.

Le 18 novembre 1994, trois cachalots mâles s'échouent sur les plages de Koksijde, ils sont suivis par un quatrième individu, le 19 novembre à Nieuwpoort. Trois de ces individus on fait l'objet d'une étude détaillée, le quatrième (Nieuwpoort) individu étant totalement putréfié. L'ensemble de ces résultats ont été publiés dans un rapport préliminaire (Annexe 3) présenté ci-après. Les conclusions principales de ce rapport indiquent clairement des niveaux élevés en cadmium, sous forme non détoxifiée (non lié au métallothionéines), dans les organes de ces animaux.

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Preliminary report of
Laboratory for Ecotoxicology and Polar Biology (V.U.B.),
Oceanology Department (Ulg),
Pathology Department (ULg).

Annexe 3

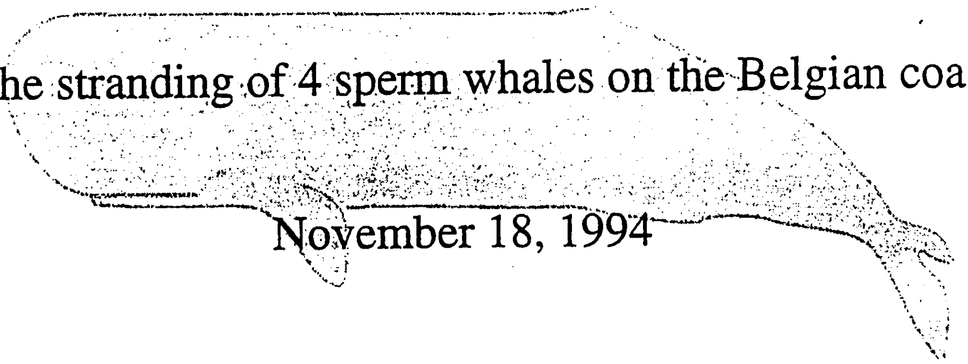
On the stranding of four sperm whales on the Belgian coast, November 18, 1994.

Annexe 3

NOT TO BE CITED WITHOUT PRIOR AUTHORIZATION OF THE AUTHORS

On the stranding of 4 sperm whales on the Belgian coast,

November 18, 1994



Preliminary report of
Laboratory for Ecotoxicology and Polar Biology (V.U.B.),
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Pathology department (ULg).

TABLE OF CONTENTS

1. SUMMARY

Abstract

Male sperm whales found dead on the coast of Belgium in November 1994 had suffered a weight deficit and displayed debilitating lesions. High levels of Cd, Hg, PCB's and DDE contamination affected all four animals. A combination of these factors could explain the stranding.

Key words
Sperm whales - stranding - pathology - ecotoxicology

Résumé

Les cachalots mâles trouvés morts à la côte belge, en novembre 1994, étaient amaigris et porteurs de lésions débilitantes. Des niveaux élevés de contamination de Cd, Hg, PCB's et DDE affectaient les quatre animaux. Ces éléments combinés pourraient expliquer l'échouage.

Mots clés
Cachalots - échouage - pathologie - écotoxicologie

Samenvatting

De mannelijke potvissen die in november 1994 dood aangetroffen werden aan de Belgische kust leden aan gewichtsverlies en vertoonden verzwakkende letsels. De vier dieren waren allen aangetast door hoge niveau's van Cd, Hg, PCB en DDE verontreiniging. Een kombinatie van deze factoren zou de aanspoeling kunnen verklaren.

Sleutel worden
Potvissen - stranden - pathologie -ecotoxicologie

2. FOREWORD

The present report was designed to provide the scientific community with a first evaluation on the stranding of 4 sperm whales in Belgium in November 1994. It is clear that work is still in process at the present date in the various laboratories involved and it is understood that some of the data presented may eventually have to be re-assessed. Contact addresses are given for additional precisions or comments.

No piece of information contained in this report may be used or reproduced without a written authorization from the authors.

The authors are endebted to those who made this work possible. The financial support was guaranteed by three grants from the "Impulse program in Marine Sciences" of the Belgian Federal Office for Scientific, Technical, and Cultural Affairs (O.S.T.C.) (contracts MS/03/031, MS/12/032, MS/12/033). Thanks are also due to those people who helped the scientific teams during the successive steps of this evaluation.

Mr. Dewulf , mayor of the city of Koksijde and the director of the Apostroff hotel, who offered his facilities to the necropsy team, the Marine Fisheries Station of the Belgian Ministry of Agriculture, and the Service of Waterways/Coast of the Ministry of the Flemish Community, deserve our very special commends and gratitude.

Prof. BOUQUEGNEAU J.-M.
Prof. COIGNOUL F.
Prof. JOIRIS Cl.

June 1995

3. PARTICIPANTS

3.1. Necropsies and toxicology

BOUQUEGNEAU J.M.(2)	Head toxicology (Cd, Cr, Cu, Zn, Fe, Pb, Ni, Ti, Se, metallothioneins, total lipids)
BROSENS L. (3)	Necropsies, parasitology
COIGNOUL F. (3)	Head pathology (necropsies, histopathology, samplings)
DEBACKER V. (2)	Toxicology
GOBERT S. (2)	Toxicology
HOLSBEEK L. (1)	Toxicology
JACQUINET E. (3)	Necropsy sperm whale #2
JAUNIAUX T. (3)	Necropsies coordination + necropsy sperm whales #1 and 4
JOIRIS Cl. (1)	Head toxicology (Hg, PCB's, pesticides, extractible lipids)
LAMBRIGTS D. (3)	Necropsy sperm whale #3
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NELLISSEN J.P.(2)	Toxicology

3.2. Administrative and technical coordinations

JACQUES T. (4)	Administration officer and on-scene coordinator of scientific teams
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Fax : 32-2-646.44.33

(6) and (7) Contact through (3)

3.5. *Additional comments*

The presence during necropsies and the valuable help from a group of 14 veterinary students interested in cetaceans (the CETO club) was most appreciated. For any additional information on this group, contacts can be made through the Department of Veterinary Pathology of the University of Liège (3).

4. CASE HISTORY

Three beached sperm whales *(Physeter macrocephalus)* were discovered on Friday November 18, 1994 between 06:00 and 07:00 local time (05:00-06:00 UT) in the tidal zone of the beach, east of Koksijde, a city located at the Belgian coast, 7 km from the French border. Stranding was considered to have occurred between 00:00 and 06:00, the same day.

A team including 3 graduate students in veterinary pathology, 3 graduate students in oceanology, and 14 undergraduate veterinary students was dispatched. They arrived on the spot at 12:00 (UT).

Local authorities forbade any sampling, except for small skin fragments, until waste disposal equipment was available at the site of necropsy.

After further delays due to tidal immersion of bodies, necropsies started on Saturday Nov. 19 at 02:00 (UT). They were simultaneously performed on the 3 bodies and lasted for 5 hours, until the tidal movement interrupted the procedure.

A fourth sperm whale was found dead on Friday November 18, 1994 (15:00 UT)in shallow water, near the beach of the city of Nieuwpoort, 8 km east of Koksijde and was towed to the beach.

Weather conditions: Fine drizzling rain and low visibility. The air temperature was 12°C, the wind was moderate to strong, blowing from the S. W., parallel to the coastline. Tidal movements were: high tide: 12:30 (UT); low tide: 18:00(UT).

5. BODY WEIGHTS

Bodies were weighted at the process plant at the time of carcasses disposal, on November 21, after partial dissection on the beach and loss of body fluids (table 1)

Table 1 : weight of carcasses reported by the processing plant (Animalia Produkten N.V., Denderleeuw)

Viscera (animals 1+2)	6,500 kg
Body weight (animals 1+2)	54,040 kg
Body weight (animal 3)	19,080 kg
Body weight (animal 4)	34,040 kg
Lower jaws (animals 1, 3, and 4)	500 kg

Calculations on total body weight were :

Sperm whales 1 and 2 :

$(54,040 + 6,500 + 330) \times 1.14 = 69,392$ kgs

Note : The correction factor 1,14 is used to compensate for loss of body fluids during dissection and transport (Lockyer, 1991).

Estimated individual weight (1 and 2) = 35,000 kgs

Sperm whale 3 :

$(19,080 + 165) \times 1.14 = 21,940$ kgs (estimate)

Sperm whale 4 :

$(34,040 + 165) \times 1.14 = 38,994$ kgs (estimate)

According to the literature, a predictive formula of normal weight can be used from measured length (Lockyer, 1991) as :

$$W = 0.0218 \times L^{2,74}$$

The normal weight derived for the Belgian whales is given in table 2 in comparison with actual weights.

Table 2 : Length, observed weight and predicted normal weight of sperm whales stranded on the Belgian coast, Nov. 18, 1994.

Animal #	Length (m)	Weight (kg)	Predicted weight (kg)
1 + 2	15.40 and 14.90	70,000	74,800
3	14.40	21,940	32,500
4	18.20	38,994	61,800

6. NECROPSIES

A summary description of carcasses, necropsies, and histopathology is presented bellow. Additional information for veterinary pathologists can be obtained on request (3).

A standardized necropsy procedure derived from the "ECS protocol for postmortem examination and tissue sampling of small cetaceans" was applied (Kuiken and Garcia Hartmann, 1991).

Samples were collected for additional evaluations (Appendix). For histopatholgy, organs and lesions were stored in 10% neutral buffered formalin. For bacteriology, intestinal segments were collected. For parasitology, intestinal content was sampled, parasites were collected and preserved in 70% ethanol. For toxicology, samples were collected and frozen. Skin samples were stored in 20% DMSO saturated with NaCl for DNA analyses. Two to four teeth from the middle of the lower jaw were collected for age determination. Body measurements were taken on each animal. The body condition was estimated using code from 1 (live animal) to 5 (mummified carcass). Photographs of both sides were taken, plus body openings and lesions. The blubber thickness was measured at the caudal insertion of the dorsal fin.

Skin, body openings (mouth, eyes, blow-hole, ears opening, genital slit and anus) were examined, and any lesions or discharges were characterized. Pictures were taken when needed.

For abdominal cavity opening, incisions were made throught the skin and the blubber. One, horizontally, at the mid height of the body, from the pectoral flipper to the anus level and two vertically, the first from the cranial part of the horizontal cut to the belly and the second from the caudal part to the anus. The skin and blubber flap was tied near the horizontal opening and the ropes were pulled for dissection of the abdominal blubber and exposure of abdominal muscles. A strip of tissue was removed and subcutaneous tissue and blubber were examined for lesions and parasites. With the same technique, abdominal muscle layers and peritoneum were removed.

Bacteriological samples were collected before handling abdominal organs. Organs, lesions and parasites were characterized, photographed and collected. The gastro-intestinal tract was examined *in situ*. The stomach was opened and the gastric content was stored. Various segments of intestine were opened and examined. After removing the digestive system, the kidneys and the liver were examined and sampled.

The diaphragm was incised and through the opening, the lung was examined and sampled.

6.1. Description of carcasses

The 3 whales of Koksijde were numbered 1 to 3, according to their location on the beach, starting from the west. All three were in the tidal zone at the eastern edge of the town (Figures 1 and 2). When first examined, animals were fresh, with no evidence of decay. Death probably had occurred not more than 6 to 12 hours before.

Sperm whale #1 (Figure 1)

The whale was a young adult male, 15.4 meter long, laying on its right side, parallel to the coastline, back to the sea, at the upper level of the tidal zone. No vestigial tooth was visible at the upper jaw. At the time of necropsy, the carcass was moderately decomposed and the penis could be extended (ECS condition code: 3) (Kuiken and Garcia Hartmann, 1991).

Sperm whale #2 (Figure 1)

The whale was a young adult male, 14.9 meter long, laying on its left side, parallel to the coastline, back to the beach, 3-4 meter lower than whale #1 in the tidal zone. Distance between animals 1 and 2 was about 50 meters. In the upper jaw, vestigial teeth were visible, 3 on the right, 5 on the left. At the time of necropsy the carcass decomposition was moderate, the penis could be extended (ECS condition code: 3) (Kuiken and Garcia Hartmann, 1991). Small round ulcers of the hard palate were observed on the mid-line.

Sperm whale #3 (Figure 2)

The whale was a young adult male, 14.4 meter long, laying on its right side, perpendicular to the coastline, tail fluke to the sea, at the middle level of the tidal zone. The distance between animals 2 and 3 was about 200 meters. No vestigial tooth was visible in the upper jaw. At the time of necropsy, the carcass was moderately decomposed and the penis could be extended (ECS condition code: 3) (Kuiken and Garcia Hartmann, 1991). Large acute ulcers of the hard palate, involving the entire cranial half of the mucosa, were observed and sampled for histopathology.

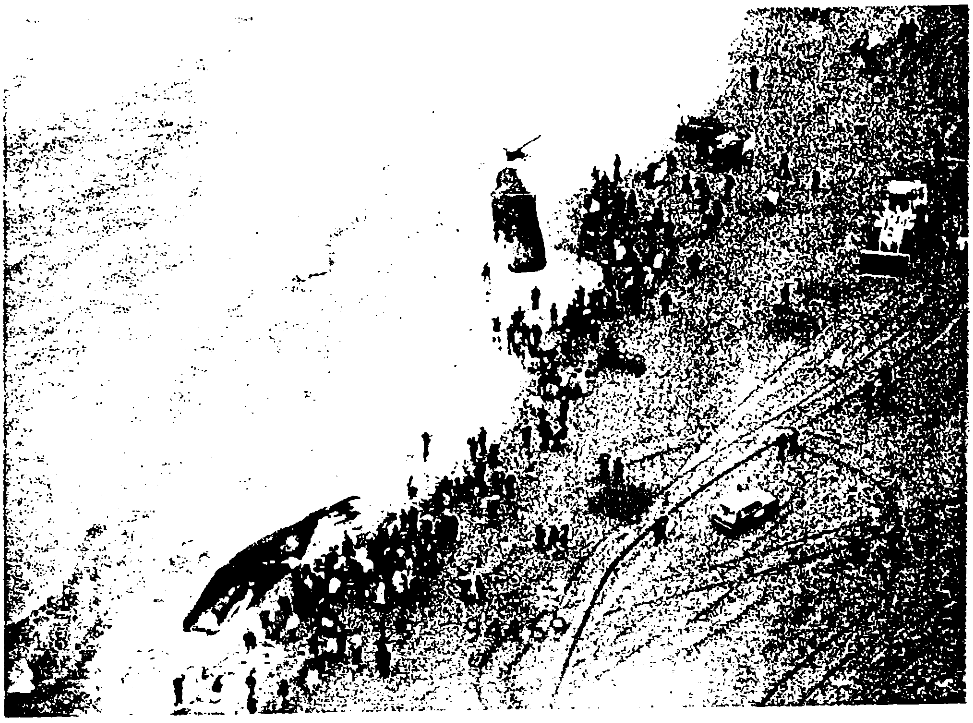

Figure 1 : Sperm whales #1 (lower left) and #2 on the beach of Koksijde, Nov. 18, 1994 (photograph E. Donnay, MUMM).

Figure 2 : Sperm whale #3 on the beach of Koksijde, Nov. 18, 1994 (photograph E. Donnay, MUMM).

Figure 3 : Sperm whale #2. Note the round white scars on the upper jaw, resulting from squid tentacles, and evenly spaced, parallel, elongated scars on the lateral aspect of the head, resulting from fights, Nov. 18, 1994 (Photograph E. Donnay, MUMM).

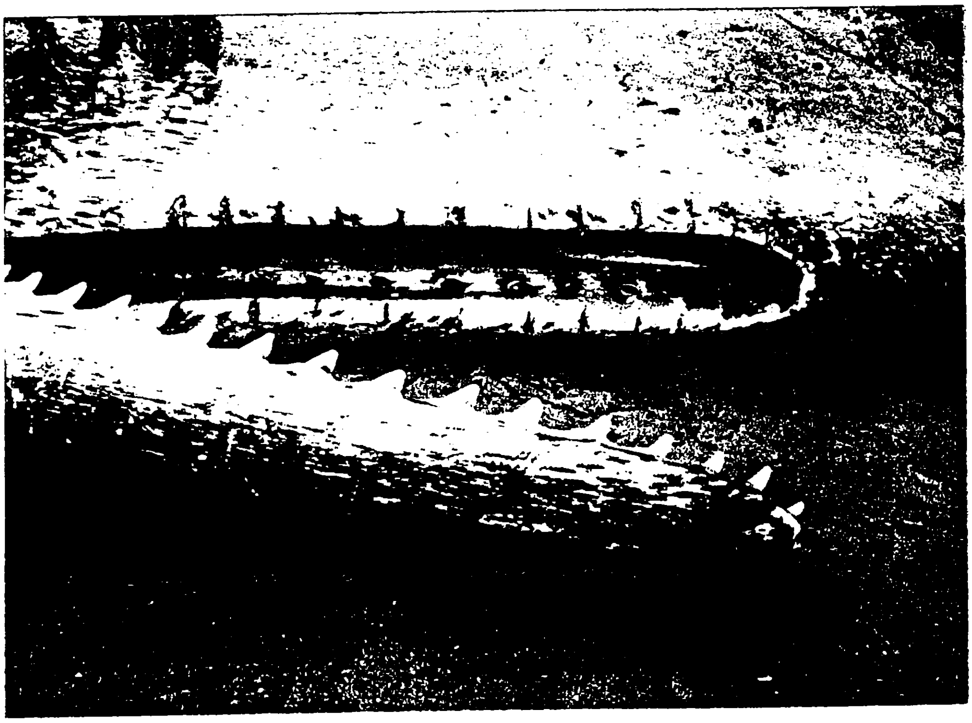

Figure 4 : Sperm whale #2. Post mortem wounds of the upper lip due to rythmic closure of the lower jaw, Nov. 18, 1994 (Photograph E. Donnay, MUMM).

Sperm whale #4

This animal, found dead at sea, off the Belgian city of Nieuwpoort, was towed on the beach of the military base of Lombardsijde. It was first examined on Saturday Nov. 19, 1994 at 03:00 PM (UT). At that time, decay was severe.

It was an adult male, 18.2 meter long. On the right upper jaw, 4 vestigial teeth were visible. The carcass was in advanced decay and the penis was extended (ECS condition code: 4) (Kuiken and Garcia Hartmann, 1991). Body openings were examined and hemorrhagic fluid dripped from the blow-hole and from the mouth. The animal was bloated.

Chronic skin lesions (parallel and round scars on the head, round scars on the tail stock) were observed on all four animals.

Skin lesions were examined on the 3 whales of Koksijde. Round scars, 4 to 5 cm diameter, that were observed on the upper jaws (Figure 3), probably resulted from the attachment of squid tentacles (Evans, 1987), squids being a normal prey of sperm whales. Longitudinal parallel scars on the head (Figure 3), probably resulted from fights between males (Evans, 1987). Those marks were separated by a more or less even distance of 10 cm, compatible with the space between adjacent teeth. A 20 cm long vertical groove on the head of whale #2 was also, most probably, a wound scar. Evenly spaced upper lip lacerations were typically post-mortem, since no bleeding was associated with the wounds (Figure 4). They resulted from the rythmic closure of the lower jaws due to water movement on the dead bodies.

Erosions were observed on all 3 animals on the lower belly, around genitalia and on the fluke (Figure 5). They probably were mechanical abrasions due to the rubbing of the stranded animals on the sand during agony. Conversely, round ulcerative lesions on the dorsal side of the tail stock resembled similar descriptions of attachement sites of sharks such as *Isistius brasiliensis* or lampreys (Evans, 1987).

Figure 5 : Sperm whale #2. Erosions around genitalia and lower belly associated with rubbing on the sand, Nov. 18, 1994 (Photograph F. Migeotte).

6.2. *Dissection*

Internal lesions observed on necropsy in animals #1, 2 and 3 included severe passive congestion of liver and kidneys, segmental congestion of intestine, mild lung passive congestion, and disseminated hemorrhages of the intestinal serosa.

On sperm whale #3, there were lesions in the outer ear canal : epithelial thickening, cells desquamation, and exudate. Ears were not examined on the other whales.

Body decay, rated as #3 body condition according to the ECS necropsy protocol, was somewhat different in the 3 sperm whales. Animal #1 appeared to be slightly fresher than #2, itself in slightly better condition than #3. This information might indicate a different time of death, #3 dying first, then #2, then #1.

No valid conclusion could be drawn from animal #4 that sat on another beach for an additional 15 hrs, was bloated at the time of sampling, and was not necropsied.

The lack of lung edema, at least in the small fragment of lung available from one whale (#1), suggested a circulatory failure rather than asphyxia. However, the lack of information on the heart and most of the thoracic cavity organs preclude any conclusive opinion on this topic.

The presence of a vestigial blow-hole in sperm whale #1 was reported before and appears to be a rare, but existing congenital anomaly in cetaceans. Also, the presence of vestigial teeth in the upper jaw of male sperm whales is described as an usual finding. It occured in 2 animals, namely whales #2 and 4.

6.3. Conclusions based on pathology

Animals #1, 2, and 3 were alive at the time of stranding, as suggested by hemorrhagic ventral abrasions.

Death occured less than 12 hours before discovery, therefore during the night of Nov. 17-18, 1994, as suggested by the lack of body decay when first examined.

The 3 animals died either simultaneously or during a short period of time, possibly #3 dying first, then #2, then #1.

Animals #3 and 4 had a severe weight deficit, compared to normal reference values, namely 32% for animal #3, and 37% for animal #4. Weight deficit was only 6,5% for sperm whales #1 and 2.

Passive congestion, observed on all 3 animals stranded at Koksijde, even in tissues located at the upper part of dead bodies confirmed an acute circulatory disturbance as the cause of death. The most likely process appeared to be cardio-vascular failure, no lesion being indicative of shock or asphyxia. This conclusion is a mere proposal and could not be confirmed due to the lack of complete dissection of the cardio-pulmonary system.

Relevant lesions were observed on animals #2 and 3, both having acute ante-mortem ulcers of the hard palate. The origin of those lesions is presently under investigation. A report will be published separately.

Ear canal lesions on whale #3 were confirmed on histopathology as being a subacute to chronic otitis. The potential extension of such lesions to the middle ear and inner ear is reported in domestic animals, with corresponding clinical signs. There was no possibility to investigate a potential extension of the lesions to the skull.

Postmortem findings were confirmed on histopathology in regard to a more severe decay in whale #3 than #1 and 2.

Parasitology and bacteriology conclusions were not significant.

7. TOXICOLOGICAL ANALYSES

7.1. Heavy metals

In order to detect possible toxic concentrations, nine heavy metals were analysed in the liver, muscle and kidney of the stranded sperm whales (table 3). Such an assessment is not easy since, on one hand, a wide range of "natural" concentrations can be encountered in a single species (in relation *e.g.* with age, sex and season) and, on the other hand, a more or less important part of toxicant may be found in the tissues bound to some ligands under a detoxified form. This is well known *e.g.* for zinc, cadmium, copper and mercury which can bind to metallothioneins (cytosolic low-molecular weight proteins with high cystein content). The speciation of the four metals has therefore been studied with respect to their binding to these proteins (tables 4, 5, 6 & 7 for zinc, cadmium, copper and inorganic mercury respectively). Inorganic mercury is moreover known to bind to selenium as non-toxic thiemannite in the lysosomes of liver and kidneys of some seabird and marine mammal species. The selenium content of both organs of the sperm whales has therefore been analysed in order to assess to which extend mercury could be stored under the thiemannite form (table 8).

Zinc, lead, nickel, cadmium, iron, chromium, copper and titanium were analysed by I.C.P.S., total mercury and selenium by flameless atomic absorption spectrophotometry and cathodic stripping voltammetry respectively. Methylmercury was determined by gas chromatography. Total lipids were estimated following the sulphophosphovanillin method. Polar lipids were extracted with HGRAMG in a 12 H Soxhlet extractor.

Tissues were homogenized and then centrifuged at 26,000 g to separate "soluble" and "insoluble" fractions. The supernatant was filtered on a LKB Ultrogel AcA 54 column. Copper, zinc , cadmium and mercury were analysed in the chromatographic fractions by atomic absorption spectrophotometry.

The fact that analyses of metallothioneins were performed on tissues deep-frozen several hours after the death of the animals requires some comments, since our technical approach implies that these proteins have not been hydrolysed and that the binding of the metals has been maintained. Fortunately metallothioneins are thermostable proteins and according to both the sharpness of the chromatographic peaks and previous observations on stranded guillemots' metallothioneins (see Bouquegneau et al, 1995), it appears that the tissues were fresh enough to assess with sufficient accuracy the actual amount of heavy metals bound to the metallothioneins.

Table 3: Heavy metal content of liver, muscle and kidney (mg/kg dw) of the sperm whales stranded on the Belgian coast, Nov. 18, 1994.

	Animal #	**Liver**	**Muscle**	**Kidney**
Zn	212	90	184	84
	213	100	237	140
	214	95	140	
	214		148	
Pb	1	2.2	0.8	1.0
	2	1.1	2.8	3.6
	3	0.9	1.6	
	4		1.4	
Ni	1	0.2	0.4	0.4
	2	0.2	0.6	0.7
	3	0.3	0.4	
	4		0.4	
Cd	1	103	1.4	225
	2	71	1.7	316
	3	64	1.0	
	4		1.9	
Fe	1	2560	393	1190
	2	2110	552	1130
	3	1990	430	
	4		590	
Cr	1	0.1	0.9	0.4
	2	0.1	0.7	0.7
	3	0.3	0.3	
	4		1.2	
Cu	1	5.3	1.9	13.4
	2	7.9	1.6	43.5
	3	6.5	1.3	
	4		2.5	
Ti	1	0.2	<0.05	0.3
	2	<0.05	1.3	1.2
	3	<0.05	<0.05	
	4		<0.05	
Hg	1	8.7	3.1	2.0
	2	60.8	4.5	1.2
	3	43.6	4.1	
	4		3.9	
MeHg	1	0.7	2.4	0.4
	2	2.4	3.6	0.2
	3	3.0		
	4		2.8	

Table 4: Zinc speciation in liver and kidney of the sperm whales stranded on the Belgian coast, Nov. 18, 1994.

	Sperm whale #1	Sperm whale #2	Sperm whale #3	Ridlington et al (1981)
Liver				
Total content (mgZn/kg fw)	28	30	33	39.8
I.F.	15.1 (54%)	15.0 (50%)	15.8 (48%)	10.6 (27%)
S.F.	12.9 (46%)	15.0 (50%)	17.2 (52%)	29.2 (73%)
MT	4.5 (16%)	4.8 (16%)	6.6 (20%)	14.0 (35%)
Kidney				
Total content (mgZn/kg fw)	26.7	45		
I.F.	6.2 (23%)	10.4 (23%)		
S.F.	20.5 (77%)	34.6 (77%)		
MT	8.9 (33%)	21.1 (47%)		

I.F.= insoluble fraction / S.F. = soluble fraction (cytosol)
MT = fraction bound to metallothioneins.

Table 5: Cadmium speciation in liver and kidney of the sperm whales stranded on the Belgian coast, Nov. 18, 1994.

	Sperm whale #1	Sperm whale #2	Sperm whale #3	Ridlington et al (1981)
Liver	A15212	A15213	A15214	
Total content (mgCd/kg fw) I.F. S.F. MT	32 19.5 (61%) 12.5 (39%) 1.9 (6%)	21 9.5 (45%) 11.5 (55%) 2.1 (10%)	22 10.3 (47%) 11.7 (53%) 2.6 (12%)	12 1 (8%) 11 (92%) 11 (92%)
Kidney				
Total content (mgCd/kg fw) I.F. S.F. MT	83 15.8 (19%) 67.2 (81%) 21.6 (26%)	101 15.2 (15%) 85.8 (85%) 54.9 (54%)		

I.F.= insoluble fraction / S.F. = soluble fraction (cytosol)
MT = fraction bound to metallothioneins.

<u>Table 6</u>: Copper speciation in liver and kidney of the sperm whales stranded on the Belgian coast, Nov. 18, 1994.

	Sperm whale #1	Sperm whale #2	Sperm whale #3	Ridlington et al (1981)
Liver				
Total content (mgCu/kg fw)	1.6	2.4	2.3	3.0
I.F.	1.2 (75%)	1.8 (74%)	1.8 (78%)	1.1 (37%)
S.F.	0.4 (25%)	0.6 (26%)	0.5 (22%)	1.9 (63%)
MT	0.1 (8%)	0 (0%)	0.1 (4%)	0 (0%)
Kidney				
Total content (mgCu/kg fw)	5.1	13.9		
I.F.	2.5 (49%)	9.0 (65%)		
S.F.	2.6 (51%)	4.9 (35%)		
MT	1.6 (32%)	2.4 (17%)		

I.F.= insoluble fraction / S.F. = soluble fraction (cytosol)
MT = fraction bound to metallothioneins.

Table 7: Mercury speciation in liver, kidney and muscle of the sperm whales stranded on the Belgian coast, Nov. 18, 1994.

	Sperm whale #1	Sperm whale #2	Sperm whale #3
Liver			
Total content (mgHg/kg fw)	3.2	19.8	14.7
MeHg	8%	4%	7%
I.F.	85%	95%	84%
S.F.	15%	5%	16%
MT	2%	<1%	<1%
Kidney			
Total content (mgHg/kg fw)	0.5	0.6	
MeHg	21%	21%	
I.F.	72%	70%	
S.F.	28%	30%	
MT	1 %	14%	
Muscle			
Total content (mg/kg fw)	0.9	1.3	1.1
MeHg	76%	79%	

I.F. = insoluble fraction / S.F. = soluble fraction (cytosol)
MT = fraction bound to metallothioneins

Table 8: Molar ratio between total Hg and Se contents in the liver, kidney and muscle of the sperm whales stranded on the Belgian coast, Nov. 18, 1994.

	Sperm whale #	Hg/Se
Liver	1	0.59
	2	0.55
	3	0.87
Kidney	1	0.09
	2	0.08
Muscle	1	0.54
	2	0.67
	3	0.49
	4	0.67

Table 9: Heavy metal content (mg/kg dw) of liver and muscle of sperm whales stranded on Belgian and North Pacific American coasts.

	Liver				Muscle
	Zn	Cd	Cu	Hg	Hg
Belgian coast (individual data)					
1994 : 1	90	103	5.3	8.7	3.1
2	100	71	7.9	60.8	4.5
3	95	64	6.5	43.6	4.1
4					3.9
1989 (1)				50.0	2.7
North Pacific coast					
mean values	124(2)	39(2)	9.4(2)		
range					(4.0 - 5.6)(3)

(1) male adult stranded alive on 12.02.89 (Joiris et al., 1991)

(2) from Nagakura et al (1974)

(3) from Ridlington et al (1981)

Few data are available in the literature which allow to assess the potential toxicity of metals contained in the sperm whales stranded at Koksijde on November 18th 1994. From table 1 and literature data about cetaceans (see Thompson, 1990) and sperm whales in particular (Ridlington et al, 1981; Nagakura et al, 1974; Joiris et al, 1991); zinc, lead, nickel, chromium and copper concentrations are to be considered low (see table 9). Mercury content of muscle was high, but in both the range of sperm whales from the North Pacific (table 9) and the sperm whale which was found stranded in 1989 along the Belgian coast. However, sperm whale #1, probably the youngest individual among the four, displaid a lower mercury concentration.

On the contrary, the cadmium content of the liver was very high, twice the figures given for the livers of the North Pacific sperm whales described by Ridlington et al (1981), but however in the range of the liver cadmium concentration of mammal species which are feeding on cephalopods, as shown in table 10.

This suggests a potential toxicity of cadmium which is strengthened by the study of the metal speciation (table 5): only a small part of the cadmium appeared to be detoxified through binding to metallothioneins (10 %) against 92 % in the livers of North Pacific sperm whales described by Ridlington. Cadmium, on the opposite to zinc and copper which were normally bound to metallothioneins (see tables 2 & 4), was potentially highly toxic for the animals.

Table 10: Mean cadmium concentration in the liver of cetaceans.

Species	(mg/kg fw)	Main diet
Harbour porpoise	0.2(1)	fish
Beluga	0.9(1)	fish
Bowhead whale	1.5(1)	crustacea/pteropods
Bottlenose whale	5.6(1)	**cephalopods**
Striped dolphin	6.3(1)	fish/**cephalopods**
Sperm whale	12.0(2)	**cephalopods**
Sperm whale	25.0(3)	**cephalopods**
Narwhal	32.0(1)	**cephalopods**
Ziphius	50.5(1)	**cephalopods**
Pilot whale	69.4(1)	**cephalopods/fish**

(1) compiled from Thompson (1990)
(2) from Ridlington et al (1981)
(3) sperm whales stranded at Koksijde on November 18th 1994.

Considering mercury (the other potentially toxic metal found in high amounts in tissues), hepatic concentration was low in sperm whale #1 (table 9) whilst it was high in the other three animals. The metal stored in the liver and kidney was mainly inorganic (less than 10 % was found under the methylated form in liver, 21 % in kidney) but, however, was not significantly bound to metallothioneins (table 7). Mercury could be mainly detoxified under the thiemannite form in the liver and kidneys of the 3 sperm whales, since the molar ratio of mercury and selenium was lower than 1 (table 8). We concluded that cadmium was potentially highly toxic for all the sperm whales. That metal is known to induce debilitation in mammals. Such a debilitation, previously quoted, is confirmed by the relatively low lipid content of liver and muscle, compared with other available data (table 11).

Table 11: Total lipid content of liver and muscle (% dw) of cetaceans.

	Liver	**Muscle**
Sperm whales stranded along the Belgian coast, Nov.18,1994		
n° 1	11	6
n° 2	12	7
n° 3	11	7
n° 4		7
mean	11	7
White-beaked dolphins stranded along the Belgian coast in		
92-93	13	11
93-94	13	5
mean	13	8
Harbour porpoise stranded along the Belgian coast in 92-93	13	15
Sei whale Bottino, 1978	22	10

7.2. *Organic xenobionts*

PCBs concentrations were determined by ECD-gas chromatography using a capillary CP-Sil 4 column, N_2 as carrier gas, a temperature programme from 60 to 270°, detector T300° after an hexane extraction and a florisil clean-up.

Data for Total PCBs (T PCBs) ranged from 1 mg/kg dw in muscle up to 17 mg/kg dw for adipose tissue. Expressed on a lipid weight basis, all tissues had concentrations exceeding 10 mg/kg. DDE concentrations up to 6 mg/kg were found.

Individual T PCBs and DDE results for muscle (M), liver (L), kidney (K) and blubber tissue (Bl), expressed respectively on a dry weight and on a lipid weight (lw) basis, are reported in table 12. DDT, Aldrine, Heptaclor were never found (detection limits on average 0.2 mg/kg lw).

Table 12: Total PCBs and DDE content in muscle (M), liver (L), kidney (K) and blubber of stranded sperm whales on the Belgian coast, Nov. 18, 1994.

Sperm whale #	tissue	mg/kg dw tot PCBs (1260)	mg/kg dw DDE	mg/kg lw tot PCBs (1260)	mg/kg lw DDE
1	M	2.6	0.3	14.2	1.9
	L	3.1	0.6	13.5	2.4
	K	29.1	14.9		
	Bl	17.5	2.4	21.0	2.9
2	M	1.6	0.2	24.0	3.2
	L	1.8	0.2	16.1	1.9
	K	7.4	0.8		
	Bl	10.9	6.3	11.7	6.8
3	M	1.0	0.1	14.3	1.6
	L	1.1	0.1	10.8	0.8
4	M	2.4	0.3	3.0	2.6
	Bl	12.2	4.8	14.6	5.8

Our results not only showed high concentrations, they also indicated a very high total load if we consider that *e.g.* 30 to 40 % of the body weight consist of adipose tissue, an equal amount of muscle tissue. A quick calculation thus leads us to total burden of 200 g organochlorine compounds for each of the sperm whales.

Reference data are not at all abundant. Because of differences in the units used, comparisons are sometimes difficult to make. We made a first selection of the literature (table 13).

Table 13: sperm whales T PCBs and DDE values in muscle (M), liver (L), kidney (K) and blubber (Bl) reported in the literature.

	n	M	L	K	Bl	unit	land
DDE	4-8 males	2.3	4.0	3.3	2.9	µg/g lw (1)	Spain
	4-6 females	2.9	6.5	2.9	4.0	µg/g lw (1)	id.
DDE	10				4.2	µg/g lw (2)	Iceland
DDE	6				3.6	µg/g fw (3)	California
DDE	12				0.22	µg/g fw (4)	Antarctic
ΣPCBs	4-8 males	24.1	30.1	9.4	9.9	µg/g lw (1)	Spain
	4-6 females	30.7	18.6	9.2	15.6	µg/g lw (1)	id.
ΣPCBs	2				39.1	µg/g lw (5)	France
ΣPCBs	10				10.5	µg/g lw (2)	Iceland

(1) Aguilar, 1983
(2) Borrel, 1993
(3) Wolman &Wilson, 1970
(4) Henry & Best, 1983
(5) Alzieu & Duguy, 1979

Any comparison with literature data without the exact ages is hard to make. Age data are usually not available but perhaps more important, our own data for the age of the animals are not yet available.

The only conclusion that can be drawn at this stage is that our data correspond very well with the levels previously mentioned (table 13). Expressed on a lipid weight basis, all data clearly exceed 10 ppm, and this for almost all individual tissues. However, the fact that the levels of organochlorine compounds found did not exceed median levels when compared with literature data does not imply that no effect would evolve from these concentrations. Organochlorine levels by far lower were found to have severe effects (teratogenic, immunodeficiency) in cetaceans and pinnipeds.

Some of our data still need to be completed; *e.g.* those on the lipid content of kidney tissue or the analysis for adipose tissue of sperm whale #3.

7.3. Conclusions based on toxicology

The four sperm whales stranded on the Belgian coast in November 1994 exhibited high levels of contamination by cadmium, mercury, PCBs and DDE.

Cadmium, which is known to induce debilitation in mammals, was found in very high concentrations, twice those previously described in the literature for sperm whales. It was not found, as it is generally the case, to be detoxified by metallothioneins. Concentrations of PCBs, DDE and mercury were in the range of literature data, but high enough to also induce severe effects such as debilitation and immunodeficiency. Mercury was not under the Hg-thionein, but could be detoxified under the thiemannite form, which should be confirmed later.

8. GENERAL CONCLUSIONS

For the present, available data on the stranding of sperm whales on Nov. 18, 1994 in Belgium, indicate that 3 animals, namely #1, 2 and 3, stranded alive at Koksijde. The 4th animal, the largest of the group, was found dead at sea and was towed to the shore during the night of 18-19 Nov.. There was no evidence of live stranding for that animal.

Severe debilitation, with weight loss of 32-37%, was evident in whales 3 and 4. The slight deficit in weight of sperm whales 1 and 2 (around 6% together) could have been shared by both animals, or represent a 12% loss in one animal. No definitive answer can be provided, since carcasses were weighted together. Blubber thickness was similar in both whales, but the validity of this parameter as an indication of weight loss in sperm whales is debatable (Lockyer, 1991).

On necropsy, there was no clear evidence of chronic lesions compatible with severe weight loss. A chronic exposure to debilitating toxics, such as xenobionts and heavy metals, is therefore a strong possibility, that is sustained by toxicology data. Total loads in cadmium, mercury, PCBs and DDE were high. Among those, it is noteworthly that mercury levels were low in sperm whale #1 only, an animal with a weight close to the normal range.

An acute disease could have prompted the stranding of the group. The most likely cause would have to be found in most severely exposed animals, namely severely debilitated #3 and 4.

Number 4, the largest, and possible leader of the group is a likely candidate. Unfortunately, it could not be necropsied. In the 3 smaller whales, #3 is to be singled out as severely debilitated, affected with large acute ulcers in the mouth and with external ear canal lesions.

However incomplete the conclusions might appear at the present time, it is coherent to imagine that the group leader (#4) died at sea and caused considerable stress to its companions, particularly one of the younger males (#3), chronically debilitated for unknown reasons, most probably stable pollutants, a condition possibly compounded by an acute severe disease. This situation may have been responsible for the stranding of the group: the shallow waters of the Belgian coastline, particularly around Koksijde, may have been fatal to the whales, animals #1 and 2 becoming disoriented by unfamiliar environment, the loss of their leader and the erratic behavior of their diseased companion (#3).

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10. APPENDIX

SAMPLES	Sperm whale #1	Sperm whale #2	Sperm whale #3	Sperm whale #4
Histopathology (1)				
diaphragm	x			
spleen	x	x		
lung	x			
kidney	x	x		
liver	x	x	x	
colon	x	x	x	
ileum	x	x	x	
gastric wall	x		x	
intestinal node	x			
palate ulcer		x	x	
blubber node		x	x	
ear duct			x	
skin			x	
Toxicology (2) (3) (4) (5)				
kidney	x	x		
muscle	x	x	x	x
liver	x	x	x	
blubber	x	x	x	x
bone (rib)	x			
ileum	x	x	x	
Bacteriology (6)				
intestine	x	x	x	
Parasitology (7)				
intestine	x	x	x	
parasites		x	x	
DNA analysis (8)				
skin	x	x	x	x
Ophthalmology (9)				
eye	x	x	x	
Age determination (10)				
lower jaw	x	x	x	x
Prey study (11)				
food remain	x	x	x	x

Samples collection on the 4 sperm whales stranded on the Belgian coast (November 18, 1994).

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(11) collection in (1)

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Annexe 4

Toxicological investigations on the sperm whales stranded in Belgium: inorganic contaminants.

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Toxicological investigations on the sperm whales stranded in Belgium: inorganic contaminants.

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Abstract - *Nine heavy metals have been analysed in the liver, muscle and kidneys of the sperm whales stranded on the Belgian coast, November 18, 1994. The concentrations of most of the studied inorganic contaminants - except mercury and cadmium - were low. The mercury content of the tissues, determined by Joiris* et al, *was high, but in the range of those found in sperm whales previously described in the literature. As shown by Joiris* et al *(this book), most of the mercury was found under an inorganic form. We found a close correlation between the mercury and selenium contents of the livers, which strongly suggests that the pollutant was detoxified under the tiemannite form, and therefore was not potentially toxic for the animals. On the contrary, cadmium was found in high concentrations, which was expectable owing to the normal diet of the species (cephalopods), but twice those previously described in the literature for sperm whales. Moreover, the metal was not found, as it is generally the case, to be bound to metallothioneins (a protein well known for its protective effect against heavy metals toxicity) and therefore most probably contributed to the debilitation of the animals.*

Marine mammals are important top predators in marine ecosystems. According to their position in the trophic networks, their long life span and their long biological half-time of elimination of pollutants, they accumulate high levels of chemicals, *e.g.* heavy metals, which have been related to the occurence of several abnormalities, debilitation, immuno-suppression, infectious diseases and impaired reproduction. All together lead to an important mortality, an increase of the frequency of strandings and a long term decline of the populations. The cause of their debilitation and/or death is generally multifactorial and related to all the environmental hazards the animals are submitted to. This has been recently shown by Swart *et al* (1994) which have demonstrated that impaired immunological functions in harbour seal were associated with chronic exposure to environmental contaminants accumulated through the marine food chain. However, the estimation of the actual toxicity of one heavy metal from its content in the tissues is not obvious: a wide range of "natural" concentrations can be encountered in a single species (in relation *e.g.* with age, sex and season), no experimental data is available on marine mammals, and it is known that they are able to detoxify part of the heavy metals contained in their tissues.

It is a matter of fact that heavy metals can be stored and detoxified by marine animals either by binding to specific proteins or by a compartmentation process within membrane-limited vesicles (see Bouquegneau *et al* (1984) and Bouquegneau & Joiris (1988)). Marine mammals are able to bind metals such as zinc, cadmium, copper and inorganic mercury to metallothioneins which are cytosolic low-molecular weight proteins with high cystein content (see *e.g.* Tohyama *et al* (1986)). Homeotherms, marine mammals have to consume large amounts of food and, owing to the important assimilation efficiency of the methylmercury contained in it, they naturally contain high levels of the pollutant and have had to develop detoxification processes of that very toxic compound, so that the accumulation of large amounts of mercuric chloride granules can be considered an adaptation to dietary mercury (fig. 1).

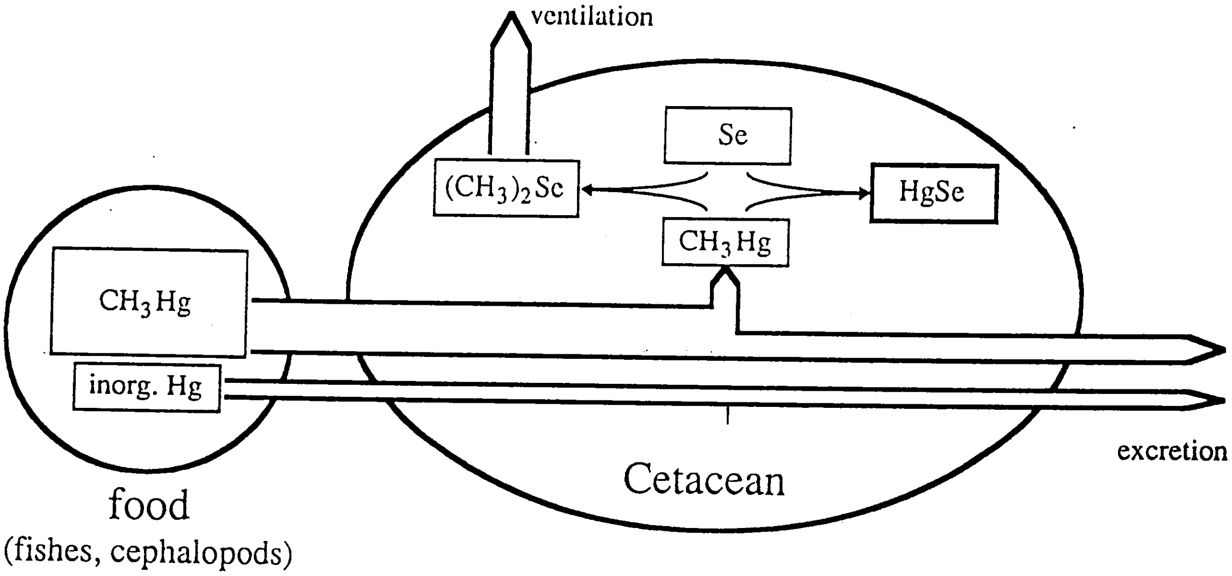

Fig. 1: fate of dietary mercury in cetaceans.

Koeman *et al* (1973) first reported a causal relationship between mercury and selenium in marine mammals. After that, Martoja & Berry (1980) have identified particles of pure tiemannite (mercuric selenide) stored in granules of the connective tissue of the liver of *Ziphius cavirostris*. Since these works, many authors have confirmed both the correlation between selenium and mercury, and the presence of tiemannite in dense intracellular granules, not only in the liver, but also in the spleen, lungs, brain, muscle and kidneys of marine mammals (see *e.g.* Nigro & Leonzio (1993)).

These detoxifying mechanisms (binding to metallothioneins, compartmentation in membrane-limited vesicles, granules) lead to high but non toxic concentrations of heavy metals in the tissues. The speciation of the metal is therefore needed to evaluate its potentially toxic concentration.

Nine heavy metals have been analysed in the liver, muscle and kidneys of the four stranded sperm whales. The speciation of cadmium, copper, mercury and zinc has been studied in respect with their binding to metallothioneins. The selenium content of liver and kidneys of the sperm whales has been analysed in order to assess to which extend mercury could be stored under the tiemannite form in the animals. Zinc, lead, nickel, cadmium, iron, chromium, copper and titanium were analysed by I.C.P.S., total mercury and selenium by flameless atomic absorption spectrophotometry and anodic stripping voltammetry respectively. Finally, in order to try to assess the debilitation of the animals, total lipids were estimated following the sulphophosphovanillin method. For the study of speciation, the tissues were homogenized and then centrifuged at 26000 g to separate soluble and insoluble fractions. The supernatant was filtered on a LKB Ultrogel AcA 54 column. Copper, zinc , cadmium and mercury were analysed in the chromatographic fractions by atomic absorption spectrophotometry.

The fact that the analyses of metallothioneins have been performed on tissues deep-frozen several hours after the death of the animals requires some comments, since our technical approach implies that these proteins have not been hydrolysed and that the binding of the metals has been maintained. Fortunately metallothioneins are thermostable proteins and according to both the sharpness of the chromatographic peaks and previous observations on stranded guillemots' metallothioneins (see Bouquegneau *et al*, 1995), it appears that the tissues were fresh enough to assess with sufficient accuracy the actual amount of heavy metals bound to the metallothioneins.

The debilitation of the stranded sperm whales, previously quoted, was first confirmed by the relatively low lipid content of the liver and muscles, compared with literature data (*e.g.* Bottino, 1978).

Few data are available in the literature which would allow to assess the potential toxicity of the metals contained in the sperm whales stranded at Coxijde on November 18th 1994. From our results and literature data about cetaceans (see Thompson, 1990) and sperm whales in particular (Ridlington *et al* (1981), Nagakura *et al* (1974) and Joiris *et al* (1991)), zinc, lead, nickel, chromium and copper concentrations are to be considered low, while mercury and cadmium levels appear to be quite high.

The mercury content of the muscle is important, but however in the range of both sperm whales from the North Pacific (Nagakura *et al*, 1974) and the sperm whale which was found stranded in 1988 along the Belgian coast (Joiris *et al*, 1991). Sperm whale n°1, probably the youngest individual among the four, displays a lower mercury concentration. The metal stored in the liver and kidneys is mainly under the inorganic form (less than 10 % was found under the methylated form in the liver, about 20 % in the kidneys, as shown by Joiris *et al* (this book)). No detectable amount of inorganic mercury was found to be bound to metallothioneins, but a close relationship was shown between selenium and mercury contents of the liver, which suggests a detoxification of methylmercury under the tiemannite form.

On the contrary, the cadmium content of the liver was very high, twice the one of the livers of the North Pacific sperm whales described by Ridlington *et al* (1981), but however in the range of the liver cadmium concentration of mammal species which are feeding on cephalopods, as shown in table 1.

species	(mg/kgFW)	main diet
harbour porpoise	0.2 (1)	fish
beluga	0.9 (1)	fish
bowhead whale	1.5 (1)	crustacea/pteropods
bottlenose whale	5.6 (1)	**cephalopods**
striped dolphin	6.3 (1)	fish/**cephalopods**
sperm whale	12.0 (2)	**cephalopods**
sperm whale	25.0 (3)	**cephalopods**
narwhal	32.0 (1)	**cephalopods**
ziphius	50.5 (1)	**cephalopods**
pilot whale	69.4 (1)	**cephalopods/fish**

(1) compiled from Thompson (1990)
(2) from Ridlington *et al* (1981)
(3) sperm whales stranded at Coxijde on November 18th 1994

Table 1: mean cadmium concentration in the liver of cetaceans

This suggests a potential toxicity of cadmium which is enhanced by the study of the speciation of the metal: only a small part of the cadmium appears to be detoxified through binding to metallothioneins (10 % of total cadmium, whilst Ridlington *et al* (1981) found 92 % of cadmium bound to the metallothioneins of the livers of sperm whales stranded in the North Pacific). This suggests that cadmium, on the contrary with zinc and copper which are normally bound to metallothioneins, is potentially highly toxic for the animals.

To conclude, the four sperm whales stranded on the Belgian coast in november 1994 exhibited high levels of contamination by mercury and cadmium. Cadmium, which is known to induce debilitation in mammals and was not, as it is usually the case, detoxified by metallothioneins, is therefore to be considered as one of the factors responsible for the stranding of the sperm whales.

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4. Liste des travaux réalisés dans le cadre du contrat

4.1. Articles.

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4.3. Mémoires.

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4.4. Posters.

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