Diensten van de Eerste Minister
Federale Diensten voor Technische, Wetenschappelijke en Culturele Aangelegenheden
Wetenschapsstraat 8, 1040 Brussel

IMPULSPROGRAMMA ZEEWETENSCHAPPEN

PROGRAMMATIE WETENSCHAPSBELEID
CONTRACT MS/03/031

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PATHOLOGIE EN ECOTOXICOLOGIE VAN ZEEVOGELS EN ZEEZOOGDIEREN IN DE NOORDZEE EN AANGRENZENDE REGIO'S

SYNTHESEVERSLAG 1 OKTOBER 1992 tot 30 SEPTEMBER 1996

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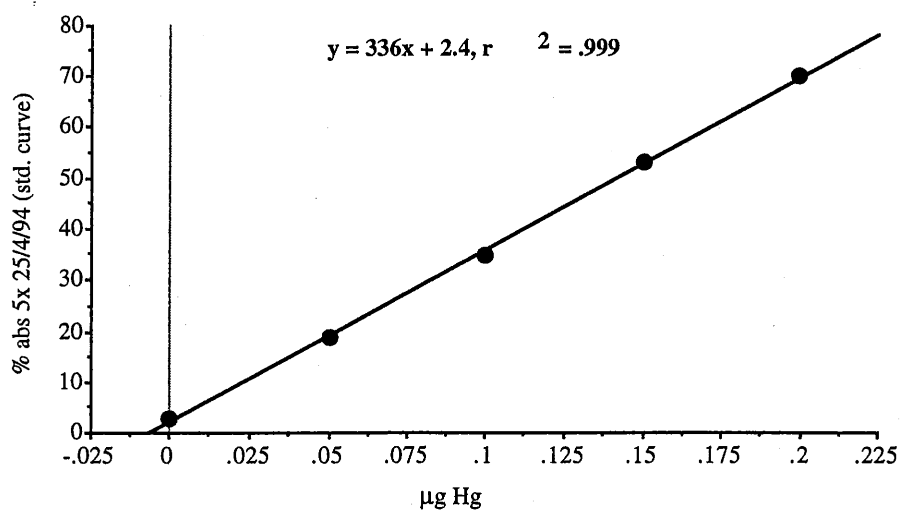
1. Inleiding

Dit syntheseverslag omvat de resultaten van vier jaar wetenschappelijk onderzoek in het kader van het Impulsprogramma Zeewetenschappen van de Federale Diensten voor Wetenschappelijke, Technische en Culturele Aangelegenheden. Dit veslag is een onderdeel van een onderzoeksproject getiteld 'Onderzoek naar de pathologie en ecotoxicologie van zeevogels en zeezoogdieren uit de Noordzee en aangrenzende regio's' waarin de inspanningen van drie ploegen gebundeld worden: het Departement Pathologie van het Instituut voor Veterinaire Wetenschappen vd ULg voor het aspect pathologie, de Dienst Oceanologie Ulg voor het deel ecotoxicologie I (Pb, Cd, Cu, Zn, Fe, Cd, Se, metallothioneinen) en het labo Ecotoxicologie en Polaire Ecologie vd VUB voor ecotoxicologie II (totaal en methyl Hg en organochloren). De resultaten vervat in dit verslag handelen enkel over het deel ecotoxicologie II.

2. Methodologie

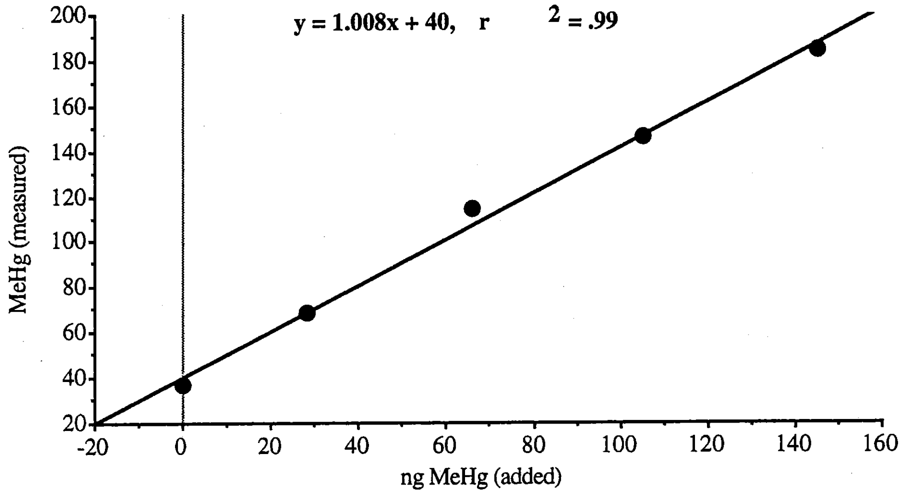
Staalname. Het verzamelen van de stalen viel onder de verantwoordelijkheid van het Vlaams Instituut voor Natuurbehoud. Onze erkentelijkheid voor de goede samenwerking gaat uit naar de heren P. Meire, J. Seys en H. Offringa alsook naar de heren J. Tavernier (KBIN), T. Jacques en J. Haelters (BMM) voor een verdere distributie van de stalen. In de praktijk werden eerst alle stalen pathologisch onderzocht (ULg Dpt. Pathologie) met opname van de biologische parameters. Tegelijk werden stalen genomen voor beide teams ecotoxicologie.

Analysen. Totaal kwik: analyse via AAS op een Perkin-Elmer MAS-50 Mercury Analyser met externe standaardcurve (figuur 1) volgens de methode beschreven door Hatch and Ott (1968), gemodifieerd zoals in detail beschreven door Joiris *et al.* (1991). Kwaliteitscontrole van de metingen gebeurt door toevoeging van verwerkte blanco's, herhaalde analyse van eenzelfde staal en analyse van een internationaal erkend referentiestaal. Absolute detectielimiet van de methode: 10 ng, overeenkomend met 10 ng/g voor een gemiddeld staal van 1g versgewicht (eventueel lagere concentraties mogelijk bij analyse van een groter staal). Coefficient van variantie voor een replicate analyse van een willekeurig staal bedroeg 8%. De methode werd getest door analyse van en vergelijk met een gecertifieerd referentiestaal met gekend ΣHg concentratie: 789 ng/g dw ± 74 (DORM-1, National Research Council Canada, Marine Analytical Chemistry Standards Programme). Een herhaalde meting van dit staal (n = 15) gaf een gemiddeld resultaat van 730 ± 30 ng/g dw, of 93 % van de target waarde. Een proef voor een eventueel optreden van een matrixeffect testte steeds negatief.



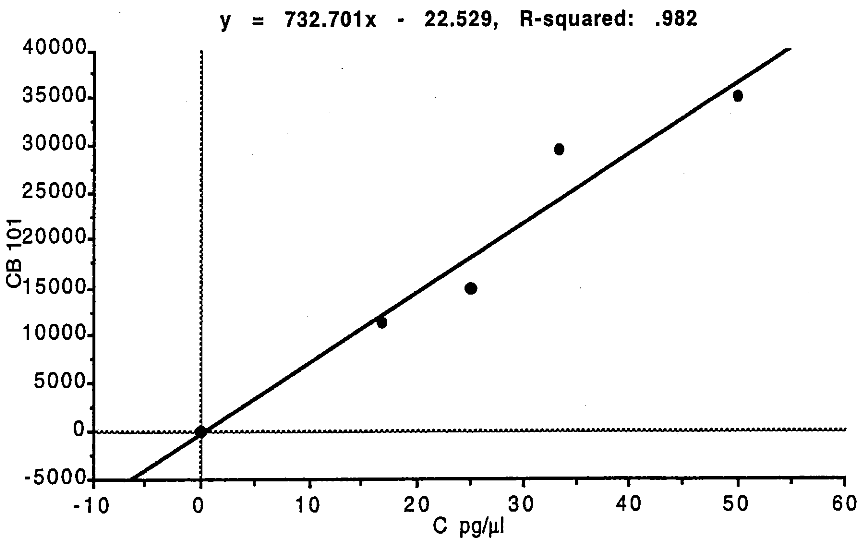
Figuur 1: externe standaardcurve ΣHg

Organisch Hg: organisch kwik wordt steeds gevonden als Methyl kwik (MeHg). Bepaling door ECD gas-chromatografie met een eenpunts externe standaardcurve op een Packard 437 GC volgend op een driestaps tolueenextractie zoals beschreven door Uthe *et al.*, 1972. Geen matrixeffect kon worden vastgesteld (figuur 2). Eenzelfde kwaliteitscontrole als voor ΣHg werd toegepast. Absolute detectielimiet van de methode: 0.02 ng MeHg (1 µl injectie) of 5 ng/g dw voor een gemiddeld staal van 1g. Herhaalde analyse (n = 12) van het gecertifieerde DORM-1 staal (731 ng MeHg/ g dw ± 60) gaf een gemiddelde waarde van 620 ± 70 of 85% van de target value. Correcties werden als dusdanig niet uitgevoerd.

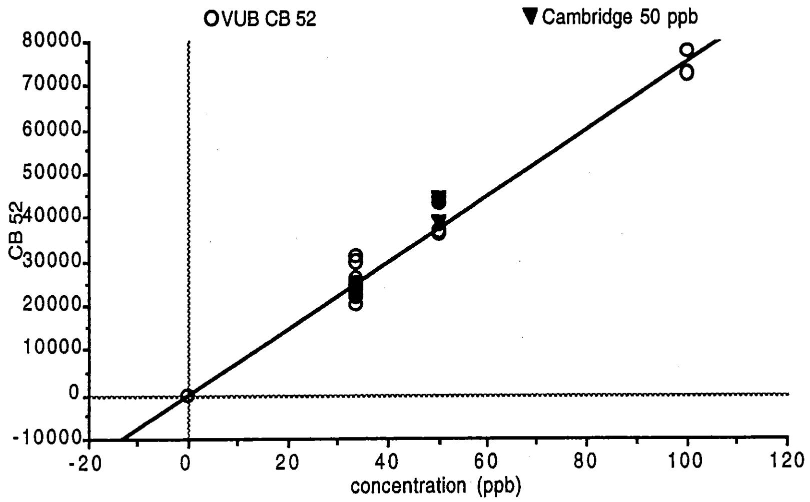


Figuur 2. Test voor matrix effect voor MeHg: toegevoegde hoeveelheid MeHg (X) en gemeten waarde (Y).

Organochloren: bepaling d.m.v. ECD gaschromatografie op een Shimadzu GC-14A met een capillair-kolom CPSil 8CB (50 m ; 0.22 mm diameter; 0.12 µm) volgend op een polaire lipiden hexaan/aceton-extractie en een florisil clean-up zoals in detail beschreven in Delbeke *et al.,* 1990. PCB data worden uitgedrukt als individuele waarden, als de som van 11 congeners (IUPAC n° 28, 31, 52, 101, 118, 153, 138, 156, 180, 170, 194 en als Total PCB na vergelijk met een aroclor 1260. Absolute detectielimiet: 5-10 pg afhankelijk van de diverse congeners. Gemiddelde detectielimiet (1g staal): 1 - 3 ng/g dw voor de individuele congeners congeners; 0.5 ng/ g dw for pesticides (heptachlor epoxide, aldrin, opDDE, ppDDT). De bepalingen gebeuren aan de hand van een externe standaardcurve, opgesteld voor elke individuele congener of pesticide (figuur 3)

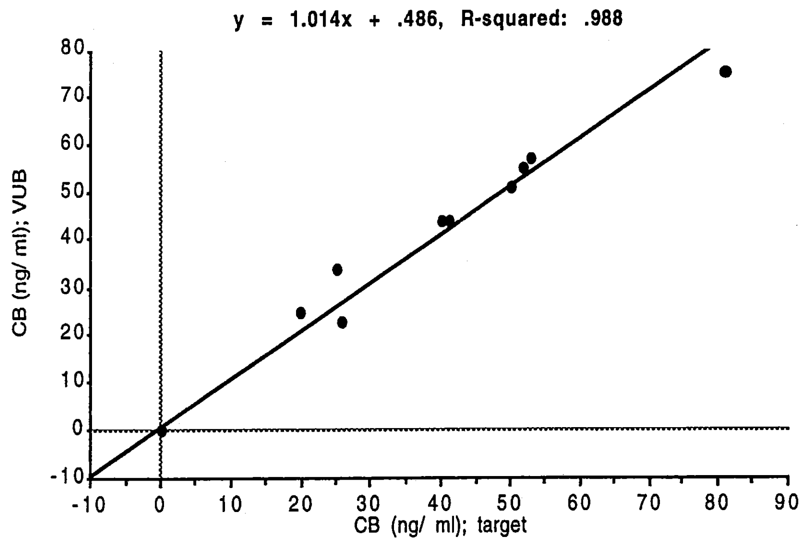


Figuur 3: Externe standaardcurve CB 101.

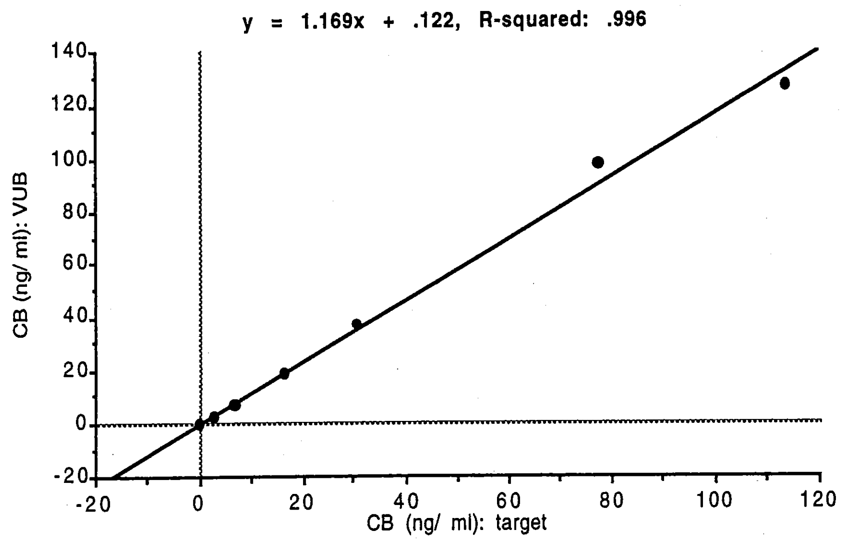


Figuur 4: vergelijk van de standaardcurve voor CB 52 (dots) en een gecertifieerde standaardoplossing (EC- 1413, Cambridge) (driehoekjes).

Kwaliteitscontrole 1 geschiedt door vergelijk van de gebruikte standaardcurven met een gecertifieerde standaard-oplossing (Cambridge EC-1413) voor een aantal van de gebruikte congeners (vb figuur 4). Kwaliteitscontrole 2: ICES PCB intercalibratie op standaardoplossingen en biologische stalen (De Boer and van der Meer, 1993). De eindbeoordeling voor de analyses uitgevoerd door ons team weken in grootteorde weliswaar af van de scherpe limiet van 5% afwijking, maar vielen binnen de 10% error grens die algemeen als goed aanvaard wordt. Figuur 6 en 7 tonen de regressierechten voor de ons team gevonden waarden (Y-as) en de ICES-targetwaarden zoals die later werden bekend gemaakt voor respectievelijk een standaardoplossing en een staal zeehonden-blubber. Bij de hier gegeven visuele voorstelling wordt de rechte door 0 geleid.



Figuur 5: ICES-intercalibratie standaardoplossing: correlatie tussen de door ons team gevonden waarden (Y) en de ICES target-waarden (X); voor 9 onderzochte individuele congeners.



Figuur 6: ICES-intercalibratie zeehonden-blubber: correlatie tussen de door ons team gevonden waarden (Y) en de ICES target-waarden (X) voor 6 onderzochte individuele congeners.

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3. Zeevogels

Gespreid over 4 opeenvolgende winters werden 287 dieren, gespreid over 13 soorten geanalyseerd voor gehaltes aan kwik en organochlore pesticiden. Gewone zeekoet is van ver de meest belangrijkste soort: teneinde een statistisch verantwoorde discussie van de resultaten mogelijk te maken werd na afloop van het eerste onderzoeksjaar samen met de andere ploegen binnen het onderzoeksproject beslist wat betreft het luik ecotoxicologie de aandacht te concentreren op de zeekoet. Zodoende werd overeen gekomen slechts andere soorten toe te voegen in het geval er onvoldoende zeekoeten voor handen zouden zijn (winters 94/95 en 95/96 bv). Als dusdanig werd impliciet aangenomen dat de intraspecifieke verschillen tussen de soorten slechts van bijkomend belang zouden bij de verwerking van de resultaten.

Species	92/93	93/94	94/95	95/96	Σ
Podiceps cristatus Fuut	-	-	-	1	1
Fulmarus glacialis Noordse Stormvogel	12	-	1	2	15
Sula bassana Jan van Gent	4	-	-	-	4
Gavia stellata Roodkeelduiker	-	-	-	2	2
Gavia arctica Parelduiker	-	-	-	1	1
Melanitta nigra Zwarte zeeëend	7	-	-	4	11
Larus argentatus Zilvermeeuw	13	-	5	-	18
Larus ridibundus Kokmeeuw	17	-	1	2	20
Larus fuscus Kleine Mantelmeeuw	-	-	1	-	1
Larus canus Strommeeuw	-	-	-	2	2
Larus sp.	-	-	-	3	3
Rissa tridactyla Drieteenmeeuw	-	-	3	-	3
Alca torda Alk	24	-	2	2	28
Uria aalge Gewone zeekoet	38+47	70	14	9	178
Σ	162	70	27	28	287

In totaal weren 287 vogels geanalyseerd voor ΣHg en methyl Hg (spier- en leverweefsel; nier wanneer de grootte van het staal dit toeliet, zie annex 1 & 2 voor detailresultaten).
Van de 178 geanalyseerde zeekoeten werden er 131 rechtstreeks gerecupereerd van het strand, de overige dieren verbleven eerst een tijd in een opvangcentrum voor olieslachtoffers alvorens in ons circuit terecht te komen. Spier, lever en nier wanneer beschikbaar van 116 zeekoeten werden geanalyseerd voor PCBs (11 congeners) en organochlore pesticiden (heptachloorepoxide, aldrine, opDDE, ppDDT).

Voor de verwerking van de resultaten van het luik ecotoxicologie werd overeen gekomen dat de coördinatie voor publicatie van algemene resultaten betreffende zeevogels zou worden waargenomen door het laboratorium voor Oceanologie, ULg. Deelresultaten werden gepubliceerd in 1994 (Bouquegneau et al.). Deze publicaties wordt niet hernomen in de Annex aangezien een latere publicatie (Debacquer et al., in press, Annex 1) deze resultaten geheel of gedeeltelijk herneemt. Een verdere gedetailleerde analyse van de resultaten voor Hg en organochloren vindt men in Annex 2 (Joiris et al, submitted).

Het gebruik van zeekoet als targetsoort was tweeërlei: enerzijds is de pathologische discussie over mogelijke doodsoorzaken en de mogelijke directe of indirecte invloed van stabiele polluenten bij dit proces. Het één en het ander wordt uitvoerig besproken in de publicatie in Annex 1. Een ander deel van de discussie, met name over de globale gezondheidstoestand van de overwinterende populatie komt duidelijk minder uit de verf. Een van de grote problemen hierbij blijft de vraag of de natuurlijke populatie op zee nu al dan niet een afspiegeling vindt in de door onze teams onderzochte dieren. Werkhypothese daarbij is nog steeds dat vooral de met olie besmeurde dieren een soort van natuurlijke populatie weergeven (sterven snel); toch blijven hieromtrent meerdere vragen nog steeds open. Aan de andere kant beschouwen we zeekoet als een goede bioindicator voor het Zuidelijke-Noordzee ecosysteem, meer specifiek bruikbaar voor een analyse in tijd en ruimte zoals besproken in Annex 2. Vooral het feit dat een opstapeling van polluenten plaatsgrijpt naarmate de overwinterende vogels langer in de Zuidelijke Noordzee verblijven trekt hierin de meeste aandacht.

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4. Zeezoogdieren

Zeezoogdieren staan onder een aanhoudende zware druk, rechtstreeks door visserij met de zeer grote problemen van bycatch, anderzijds door pollutie. Toch zijn er aanwijzingen dat, alleszins wat betreft bruinvis en mogelijk ook gewone zeehond, een toenemen van de populatie in het komend decennium meer dan aannemelijk is. Dit is echter niet noodzakelijk een gevolg van een verbeterde algemene situatie in het Belgische deel van de Zuidelijke Noordzee; een areaaluitbreiding van meer noordelijke populaties van bruinvis is hiervan minstens de rechtstreekse oorzaak (Baptist et al, 1997). Ook de recente strandingen in relatief grote aantallen van potvissen langs de kusten van de Noordzee moet op populatieniveau niet noodzakelijkerwijs als negatief gezien worden; dergelijke strandingen kunnen eventueel even goed een indicatie zijn voor een herstel op wereldschaal van de populaties van potvis.
Aan de andere kant werden zeer hoge concentraties van stabiele polluenten vastgesteld in de weefsels van vooral tandwalvissen (Subramanian et al., 1987; Subramanian et al., 1988; Joiris et al, 1991; Tanabe et al., 1994; Kuehl and Haebler, 1995 a.o.) en bestaat er een consensus over de negatieve invloed van stabiele polluenten op de afweermechanismen en voortplanting. Ons onderzoek kadert dan ook in een groter internationaal kader betreffende de impact stabiele polluenten op de populaties van zeezoogdieren

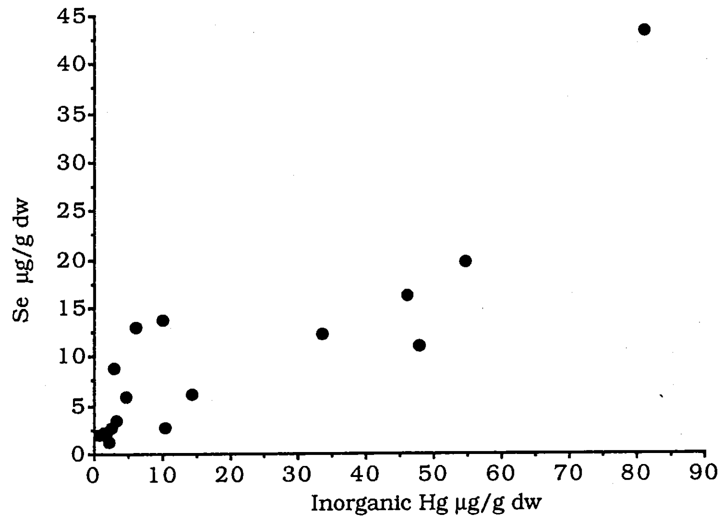
Metingen voor Totaal en Methyl Hg alsook voor organochloren werden verricht op tekens 4 weefsels (spier, lever, nier en vetweefsel) van in totaal 16 dieren:

3 *Phocoena phocoena* Bruinvis
1 *Lagenhorhynchus acutus* Witflankdolfijn
3 *Lagenorhynchus albirostris* Witsnuitdolfijn
1 *Globicephala melaena* Griend
7 *Physeter macrocephalus* Potvis
1 *Phoca vitulina* Gewone zeehond

In het geval van potvis betreft het 4 dieren gestrand op de Belgische kust, aangevuld met 3 dieren uit Nederland. Complementair aan de afspraken betreffende coordinatie van resultaten voor ecotoxicologie van de zeevogels werd overeen gekomen dat de coordinatie voor de zeezoogdieren zou worden waargenomen door het laboratorium voor Ecotoxicologie en Polaire Ecologie van de VUB. Resultaten worden weergegeven in Annexen 3 (Bouquegnequ et al., 1996) en 4 (Joiris et al.,in press) Een verdere gedetailleerde publicatie betreffende de stranding van deze zelfde potvissen is in voorbereiding.

Publicatie van de resultaten voor de overige 9 individuen (5 soorten) is op dit ogenblik weinig opportuun. In een later stadium zal deze discussie van de resultaten van deze reeks toegevoegd worden aan grotere reeksen, eventueel buitenlandse stalen. Ingeschakeld in een grotere reeks van stalen zullen zij pas dan een wetenschappelijke meerwaarde krijgen.

Zoals reeds beschreven in de literatuur een verband aangetoond tussen kwik en selenium in zeezoogdierstalen. (Martoja and Berry, 19780; Hansen et al, 1990; Paludan-Muller et al. 1993; Kuehl and Haebler, 1995) Ook voor onze reeks van zeezoogdieren, samengevoegd zonder onderscheid van soorten is er een duidelijk verband tussen de concentraties van Hg (VUB) en Se (ULg) vast te stellen in de lever van de dieren. Dit fenomeen duidt op een demethylatieproces waarna HgSe tiammaniet onbeperkt, gedetoxifieerd en onder gegranuleerde vorm opgestapeld blijft in het leverweefsel.



Figuur 7: correlatie Hg/Se in leverweefsel van zeezoogdieren gevonden aan de Belgische kust (6 soorten).

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6. Samenvatting.

Binnen het luik Ecotoxicologie II van het onderzoek naar de pathologie en ecotoxicologie van zeevogels en zeezoogdieren van de Noordzee en aangrenzende regio's werden de weefsels van 287 zeevogels en 16 zeezoogdieren onderzocht voor concentraties totaal Hg, methyl Hg en organochloren (PCBs en pesticiden).

Wat betreft het luik zeevogels werd al in een eerste periode van het onderzoek overeen gekomen alle aandacht toe te spitsen op de gewone zeekoet als indicatorsoort (grote aantallen, strikt pelagische soort, vergelijk met bestaande resultaten voorgaande jaren). Na een eerste onderzoek van de resultaten kwamen beide teams ecotoxicologie (VUB-ULg) tot de conclusie dat een tijdelijk verblijf van de dieren in vogelopvangcentra de uiteindelijke concentraties in de weefsels kan wijzigen. Een gevolg hiervan was dat dit type van dieren uitgesloten werd voor verdere discussie en bijgevolg ook geweerd werd bij verdere staalname.

Een vergelijk van de resultaten voor pathologie en ecotoxicologie legde duidelijk een verband tussen verhoogde concentraties voor Hg en PCBs en een algemene staat van verzwakking waarin de vogels gevonden werden. Olievervuiling blijkt nog steeds één van de belangrijkste oorzaken van sterfte te zijn bij de in de Zuidelijke Noordzee overwinterende vogelpopulaties. Precies deze olieslachtoffers gelden in onze studie als een soort van natuurlijke controle aangezien algemeen aanvaard wordt dat zwaar gecontamineerde vogels snel sterven. Met het leggen van een mogelijke link tussen algemene lichaamsconditie en doodsoorzaak aan de ene kant en concentraties stabiele polluenten aan de andere, werd aan een eerste opzet van het project voldaan. Een verdere analyse van de gegevens voor Hg en organochloren leidde tot de vaststelling dat er relatief grote verschillen gevonden worden tussen de verschillende jaren. Dit fenomeen blijkt echter niet significant en is uiteindelijk slechts een gevolg van een verschillende spreiding van de staalnames over de verschillende jaren. Als enig significant fenomeen komt een opstapeling met de verblijftijd in de Zuidelijke Noordzee naar voor: de gemeten concentraties in de weefsels stijgen evenredig met de verblijftijd in de Zuidelijke Noordzee. Complementaire zomergegevens voor de Noordelijke broedgebieden geven een tegengestelde beweging van met de tijd dalende concentraties weer zodat de lage concentraties bij aankomst in de Zuidelijke Noordzee binnen eenzelfde logica kunnen verklaard worden. De belangrijkste conclusie is dat Zeekoet een goede indicatorsoort is voor het meten van een locale contaminatiegraad, maar dat voor een vergelijk van resultaten in de tijd gestandaardiseerd moet worden voor verblijftijd in een ecosysteem.

Onder het luik zeezoogdieren ging een groot deel van de inspanning naar een grondige verwerking van de resultaten voor de op de Belgischeen Nederlandse kust gestrande potvissen. Gelet op de veel kleinere staalname is het veel moeilijker om tot algemene conclusies te komen en als dusdanig volledig de doelstellingen van het project in te vullen. Een analyse van de resultaten voor de ecotoxicologie geeft aan dat alle dieren onder een hoge pollutiedruk staan; het inschatten van mogelijke impact op het individueel niveau blijft echter moeilijk.

Diseases of Aquatic Organisms, in press.

Annex 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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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).

gure 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.

Acknowledgements

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

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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.

Table 1

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

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

Table 2

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

	Liver Beach	Liver Centre	p =	Kidney Beach	Kidney Centre	p =	Muscle Beach	Muscle 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 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

	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 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

	Age: Class I	Age: Class II	Sex		Cachexia		Gastro-enteropathy		Oiling		
	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 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

	Age Class I Juv. + Imm.	 Class II Adult	Sex Male	 Female	Cachexia -	 +	Gastro-enteropathy -	 +	Oiling no	 E. + I.	 E.
Muscle											
Cu	18 ± 6 18 *(77)*	16 ± 4 16 *(41)*	18 ± 6 18 *(76)*	18 ± 5 17 *(53)*	18 ± 5 18 *(37)*	18 ± 6 18 *(108)*	17 ± 5 17 *(56)*	19 ± 6 18 *(89)*	20 ± 7 19 *(65)*	16 ± 4 16 *(41)*	18 ± 5 18 *(38)*
Zn	61 ± 15 59 *(77)*	58 ± 11 57 *(41)*	58 ± 16 55 *(76)*	61 ± 11 61 *(53)*	53 ± 12 52 *(37)*	62 ± 14 62 *(108)*	57 ± 13 55 *(56)*	62 ± 15 61 *(89)*	63 ± 15 63 *(65)*	56 ± 11 54 *(41)*	59 ± 15 55 *(38)*
Fe	693 ± 292 643 *(77)*	612 ± 130 584 *(41)*	688 ± 297 641 *(76)*	649 ± 131 640 *(53)*	586 ± 145 580 *(37)*	697 ± 260 645 *(108)*	646 ± 238 599 *(56)*	683 ± 242 663 *(89)*	711 ± 264 663 *(65)*	601 ± 107 586 *(41)*	674 ± 289 648 *(38)*
Cd	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl
Total Hg	2.2 ± 1.1 1,9 *(89)*	2.0 ± 1.2 1,7 *(49)*	1.6 ± 0.9 1.4 *(20)*	2.3 ± 1.4 1.9 *(28)*	2.0 ± 0.8 2.1 *(39)*	2.1 ± 1.2 1,7 *(122)*	2.2 ± 1.3 2.0 *(62)*	2.0 ± 1.0 1.7 *(99)*	1.9 ± 0.8 1,8 *(72)*	2.5 ± 1.3 2.3 *(49)*	2.0 ± 1.3 1.5 *(41)*
Org. Hg	1.7 ± 0.8 1,6 *(77)*	1.5 ± 0.9 1,3 *(39)*	1.2 ± 0.8 1,0 *(15)*	1.7 ± 0.9 1,4 *(23)*	1.5 ± 0.7 1.5 *(33)*	1.7 ± 0.9 1,4 *(101)*	1.7 ± 1.0 1,4 *(49)*	1.6 ± 0.8 1,4 *(85)*	1.5 ± 0.6 1,4 *(64)*	1.8 ± 0.9 1,5 *(35)*	1.6 ± 1.1 1.3 *(36)*
Inorg. Hg	0.4 ± 0.4 0,3 *(77)*	0.4 ± 0.4 0,2 *(39)*	0.3 ± 0.3 0,2 *(15)*	0.4 ± 0.5 0,3 *(23)*	0.4 ± 0.4 0,3 *(33)*	0.4 ± 0.4 0,3 *(101)*	0.4 ± 0.4 0,3 *(49)*	0.4 ± 0.4 0,3 *(85)*	0.3 ± 0.4 0,2 *(64)*	0.6 ± 0.4 0,5 *(35)*	0.3 ± 0.4 0,2 *(36)*
Sum PCB	1.8 ± 1.4 1,3 *(68)*	2.6 ± 2.1 2,1 *(40)*	1.9 ± 0.9 1,6 *(17)*	3.1 ± 2.5 2,5 *(22)*	2.5 ± 2.3 2.0 *(31)*	2.0 ± 1.5 1,6 *(99)*	2.4 ± 1.9 1,9 *(46)*	2.0 ± 1.7 1,6 *(84)*	1.7 ± 1.4 1,3 *(68)*	2.9 ± 2.5 2,0 *(22)*	2.4 ± 1.7 2,0 *(39)*
Total lipids	0.08 ± 0.04 0,08 *(59)*	0.11 ± 0.08 0,08 *(33)*	0.11 ± 0.09 0,08 *(61)*	0.09 ± 0.05 0,08 *(42)*	0.13 ± 0.11 0,11 *(32)*	0.09 ± 0.05 0,08 *(87)*	0.12 ± 0.11 0,08 *(43)*	0.09 ± 0.04 0,08 *(76)*	0.09 ± 0.05 0,08 *(65)*	0.09 ± 0.05 0,07 *(17)*	0.13 ± 0.11 0.10 *(36)*
Polar lipids	0.04 ± 0.02 0,03 *(68)*	0.05 ± 0.04 0,04 *(40)*	0.06 ± 0.033 0,04 *(17)*	0.04 ± 0.03 0,03 *(22)*	0.07 ± 0.04 0,05 *(31)*	0.04 ± 0.01 0,03 *(99)*	0.05 ± 0.04 0,04 *(46)*	0.04 ± 0.02 0,03 *(84)*	0.03 ± 0.01 0,03 *(68)*	0.05 ± 0.03 0,04 *(22)*	0.05 ± 0.04 0,04 *(39)*

Table 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.

able 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	Homoya North. Norway	20.0 ± 2.9	86.7 ± 14.9	3.1 ± 1.1	1.9 ± 0.4	Wenzel and Gabrielsen, 1994
	n = 66	*winter 1989 to 95*	*Belgian coast*	*51 ± 16*	*142 ± 34*	*2.6 ± 2.0*	*5.3 ± 1.9*	*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	Homoya 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 = 61	*winter 1989 to 95*	*Belgian coast*	*29 ± 11*	*174 ± 41*	*8.2 ± 7.5*	*4.0 ± 1.5*	*this study*
Muscle	n = 24	April to Nov. 1988	Northwest Scotland	range 10.2 - 14.0	range 20.9 - 26.0	nd	range 0.5 - 1.8	Stewart et al., 1994
	n = 10	summer 1992 and 1993	Homoya North. Norway	19.2 ± 0.9	49.3 ± 3.3	0.2 ± 0.1	0.4 ± 0.1	Wenzel and Gabrielsen, 1994
	n =	*winter 1989 to 95*	*Belgian coast*	*20 ± 7*	*63 ± 15*	*<dl*	*1.9 ± 0.8*	*this study*

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

Fig. 1

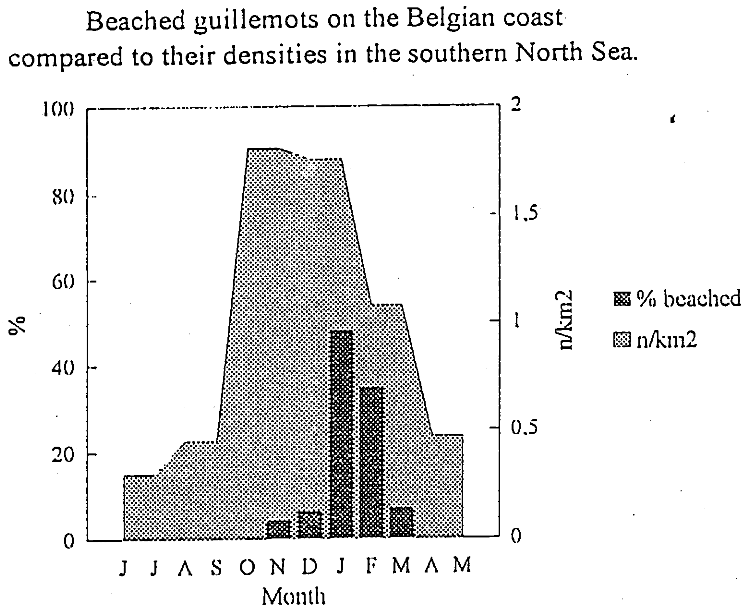


Figure 1: Overall stranding (percentage of total number) of guillemots (this work) compared to their densities (number/km^2) in the southern North Sea (Camphuysen and Leopold, 1994).

Fig. 2

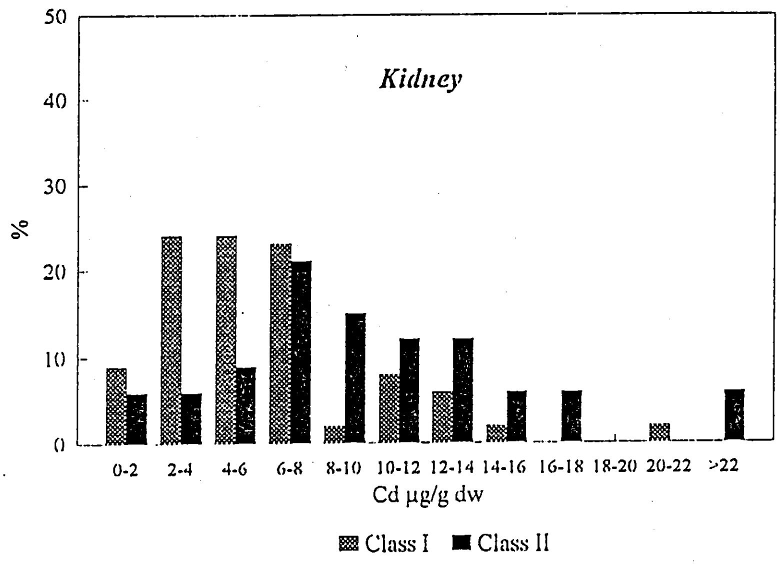
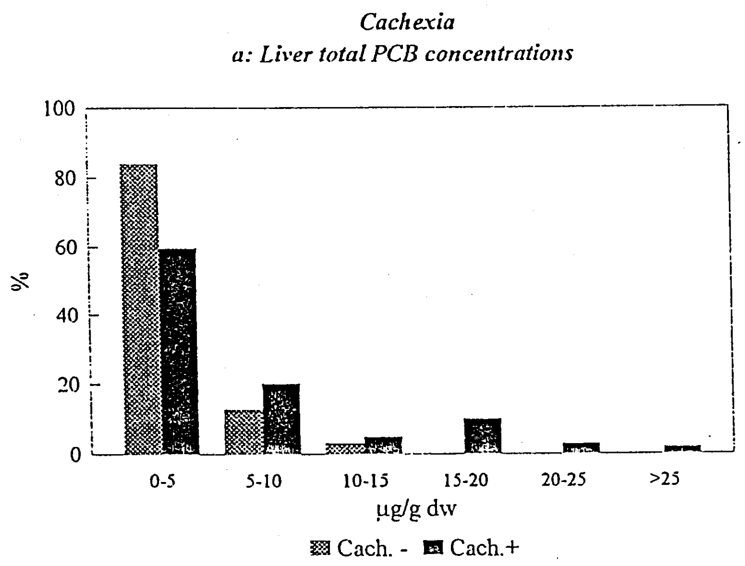


Figure 2: Relative distribution of Cd concentration for age class I (juvenile and immature) and age class II (adult) in kidney of *Uria aalge* found dead on the Belgian coast.

Fig. 3 a and b



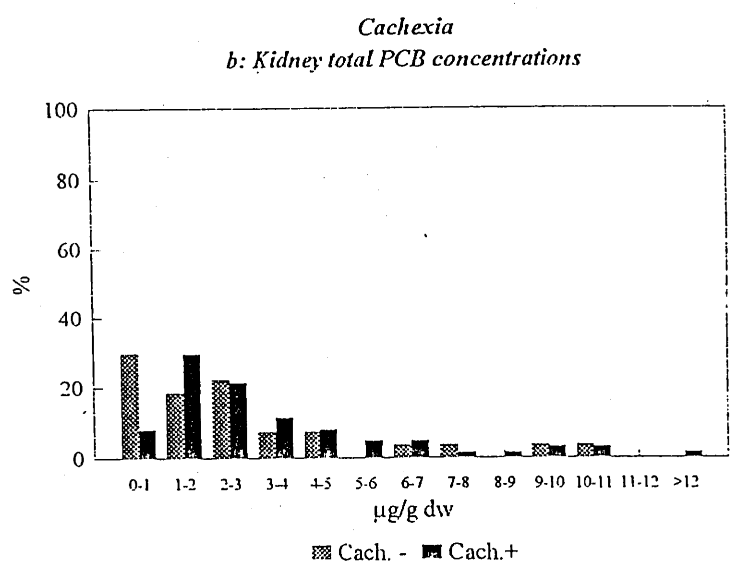


Figure 3 a and b: Relative distribution of PCB concentrations for non cachectic (Cach.-) and cachectic (Cach.+) birds in liver and kidney of *Uria aalge* found dead on the Belgian coast.

Marine Pollution Bulletin, submitted.

Annex 2

Increase of organochlorines and mercury levels in common guillemots Uria aalge during winter in the Southern North Sea.

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26/3/97
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Increase of Organochlorines and Mercury Levels in Common Guillemots *Uria aalge* during Winter in the southern North Sea.

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Beached seabirds, mainly common guillemots *Uria aalge,* were collected on the Belgian coast during winter from 1990 to 1995. Concentrations of total and organic mercury, and of organochlorines (PCBs and pesticides) were determined in muscle, liver and kidney. They were high compared with summer data (up to one order of magnitude), and increased during winter. This increase is not due to changes of total body weight nor polar lipid content, and thus reflects an actual increase of the seabirds' contamination while wintering in the southern North Sea. The observed annual cycle can be understood by assuming differences in prey contamination: higher during winter in the southern North Sea ecosystem than during summer in Atlantic water ecosystem.

In southern North Sea water, the summer density of common guillemots *Uria aalge* is very low, while winter density increases by one order of magnitude (Joiris, 1972a; 1978; 1983): recalculated as densities, figures are 0.4 and 3.2 individuals per km^2 respectively. This wintering population consists mainly of sub-adults to be recognised by their winter plumage, while the adults, already moulted into summer plumage, already concentrate in North Atlantic water closer to their breeding colonies (Joiris, 1972a, 1978, 1983). During October - November, about 235.000 guillemots are wintering in the 130.000 km^2 southern North Sea (Camphuysen and Leopold, 1994), or 1.8 guillemots/ km^2. An important at-sea mortality follows and, as a consequence, a peak in the numbers of beached birds. Most of the beached guillemots were oiled (55%) while at sea, the number of oiled seabirds was estimated as 0.5 to 1% (Joiris, 1972b).

These carcasses were used in the framework of a coordinated pathological and toxicological study of dead seabirds and marine mammals, aiming at the determination of their health status, contamination and possible causes of death. An overview of these data has been published already (Debacker *et al.,* 1997). The present paper deals with a detailed analysis of results on organochlorines and mercury concentrations.

Material and methods

Beached seabirds, mainly guillemots were collected from 1990 to 1995 during systematic winter counts along the Belgian coast (P Meire, J Seys and H Offringa, Flemish Institute for Nature Conservation). The carcasses were deepfrozen, and later analysed in the laboratory; putrified specimens were discarded while living individuals were treated in rehabilitation centres where the majority eventually died and were examined as well (Table 1). More details on these samples can be found in Debacker *et al.* (1997).

Organochlorines (PCBs and pesticides) were determined by liquid gas chromatography (Shimadzu GC-14A) with capillary column and electron capture detector (detailed description in Delbeke *et al.,* 1990). PCB data were expressed as the sum of 11 congeners (IUPAC n° 28, 31, 52, 101, 118, 153, 138, 156, 180, 170, 194) by comparison with an external standard solution. Detection limit for a 1 g sample is 1 to 3 ng/ g dry weight (dw) for individual congeners (15 ng/ g dw for Σ congeners) and 0.5 ng/ g dw for pesticides.

Total mercury (ΣHg) was determined by atomic absorption spectrometry (MAS-50 Mercury analyzer, Perkin-Elmer) after mineralisation of fresh samples with sulfuric acid and oxydation of the Hg to Hg^{++}. After reduction with stannous chloride, the volatile Hg° is bubbled into the closed system of the analyzer and the absorption measured. The method (Hatch and Ott, 1968, modified by Bouquegneau, 1973), was already described with more detail, including intercalibration, reproducibility test and test for matrix effect (Joiris *et al.*, 1991). Detection limit for a 1 g sample is 15 ng/ g dw.

Organic Hg was determined by gas liquid chromatography with electron capture detector (Packard 437)(Uthe *et al.,* 1972). For more detail, see Joiris *et al.* (1991). Detection limit for a 1 g sample is 8 ng/ g dw.

Polar lipids were extracted with a hexane - acetone mixture (135/15) for 10 hr at 75° C in a Soxhlet apparatus; the extracted lipids were weighed after evaporation of the solvent.

The obtained results did not show a normal distribution, and significancy was looked for by using non parametric tests: Mann-Witney and Kruskall-Walis for 2 data sets and more respectively.

TABLE 1

Number of common guillemots *Uria aalge* beached on the Belgian coast during winter, included in this study.

a. found dead on the beach:

winter	*1989-90*	*1990/91*	*1991/92*	*1992/93*	*1993/94*	*1994/95*	*total*
period:							
Nov. - Dec.		9	0	9	0	1	19
January		2	2	23	23	8	58
February	16	0	9	5	38	2	70
March	8	0	0	1	8	0	17
total	24	11	11	38	69	11	164

b. after stay in rehabilitation centres:

winter	*1989-90*	*1990/91*	*1991/92*	*1992/93*	*1993/94*	*1994/95*	*total*
period:					*	*	
Nov. - Dec.		16	1	5	0	0	22
January		7	14	18	0	0	39
February	12	1	17	10	1	3	44
March	31	0	1	1	0	0	33
total	43	24	33	34	1	3	138

** Fom 1993/94 on, it was decided not to treat individuals from rehabilitation centres any longer.*

Results and discussion

Substantial significant differences were noted between the concentrations of Hg and organochlorines in guillemots found dead on the beach and individuals having stayed in rehabilitation centres, some of these showing higher levels of PCBs, DDE and Hg ($p < 0.01$)(Figs. 1 - 3) and body weight significantly lower (600 g instead of 700 g, $p < 0.01$). Since we did not find any explanation for this observation, and because it could be an artifact due to human intervention during treatment in the centres, these results had to be excluded and all data presented further concern only individuals found dead on the beach (Table 1a).

In comparison with summer total body weights of about 1 kg, beached guillemots collected along the Belgian coast during the winter showed a clearly lower weight of about 700 g, with median values of 680 g for the "cachectic" individuals and 770 g for the "normal" ones (Table 2). Since this observation also concerned the oiled individuals, especially the ones internally oiled for which a rapid death by oiling is assumed to take place, one may consider that the lower weight concerns the whole wintering population, not only the individuals dying from starvation or other stress. This is confirmed by the low weight registered for guillemots beached during winter in the Dutch coastal zone, while in Scotland they still weigh 1 kg in November (Table 2).

Within the winter period, no further decrease of weight was detected for median values (Fig 4), even if a slight trend could be detected with the disappearence of the heavy individuals still having a weight of 1 kg in November - December, and later on the disappearence of the individuals with very low body weight (less than 600 g) in March: the few "heavy" guillemots seem to loose weight during winter while the most cachectic ones might die.

TABLE 2

Body weight of common guillemots *Uria aalge* beached along the Belgian coast; comparison with other studies (n = number of individuals).

	n	total body weight (g) median	min. - max.	reference
Belgian coast, winter:				this study
all	*162*	700	440 -1180	
non-cachectic	*38*	770	580 - 1180	
cachectic	*122*	680	440 - 1100	
non-oiled	*71*	680	440 - 1180	
oiled: externally	*41*	700	560 - 1120	
externally + internally	*49*	715	560 - 1100	
Dutch coast, winter	*66*	706 (79)*		Camphuysen, 1989
Scotland, November	*25*	1031 (15)*		Furness et al., 1994
N Scotland, summer	*82*	940 (14)*		Jefferies & Parslow, 1976
Wales, summer	*24*	862 (36)*		Birhead, 1976

* *mean & standard deviation.*

Lipid content was depending on total body weight, the most cachectic individuals having the lowest polar lipids level in muscle (Fig. 5). During the winter, however, no further decreasing trend in lipid content was detected (Fig. 6).

PCB data were expressed as the sum of 11 congeners (∑congeners), but their pattern could also be compared with standard mixtures Aroclor 1254 and 1260, in order to allow possible comparison with other (older) data expressed as standard mixture ("total" PCBs): the pattern was much closer to 1260 than 1254, both visually and as reflected by a higher correlation coefficient (Fig. 7), so that the results could also be expressed as standard mixture1260. This, however, does not mean that the seabirds actually contain Aroclor 1260: long-term effects of small differences in stability and/ or liposolubility of the congeners could also have led to a pattern mimicking the one of a standard mixture. All data presented here are expressed as ∑congeners, accounting for 35 % of "total" PCBs if calculated as Aroclor 1260.

PCB concentrations we determined in guillemots during winter with median values of 3 to 8 µg/ g dw in liver and 1 to 4 in muscle (Table 3) were clearly higher than the ones recorded during summer between 1970 and 1972 with mean values of 1.1 µg/ g dw in liver and 0.7 in muscle (Bourne and Bogan, 1972), but these differences could concern both a seasonal effect (summer versus winter) and a possible increase of PCB contamination with time.

Moreover, an increase of PCB concentration was noted in the different tissues during the winter: ∑congeners levels increased in liver from 3 µg/ g dw in November - December to 8 in March ($p < 0.01$), from 1 to 4 in muscle ($p < 0.01$) and from 0.5 to 2 in kidney (non - significant, small sample)(Table 3; Fig. 8). This increase is reflected as an increase of PCBs per lipid weight as well, but is not due to an important decrease of weight, nor of polar lipids (see higher)(Table 3). These data thus reflect an actual increase of PCB concentration and of total body burden in guillemots while wintering in the southern North Sea area, and are not due to starvation or stress, nor a decrease of lipids leading to a remobilisation.

Other factors were tested as possible causes for variations in PCB concentration, but provided non-significant or limited differences, or differences in some tissues only: sex, age, cachexy, oiling (external or external + internal), presence of gastro-enterital hemorrhagies, etc. (see Debacker *et al.,* 1997). Among them, cachexy deserves special attention: cachectic individuals generally have higher contaminants concentration in the liver, which can entirely be explained by the decrease of lipid content in muscle, causing a remobilization of the liposoluble contaminants, and an incease of their concentration in liver. As a consequence, the liver - to - muscle ratio of PCB concentration is higher as well. These values, however, did not increase with time and thus do not provide any explanation for the increasing concentrations observed during the winter.

Among the organochlorine pesticides analyzed, following were not detected in any sample: HCB, opDDD, pp'DDD, pp'DDT, endrin, trans-heptachlor epoxide, heptachlor, methoxychlor, Mirex. The concentration of others were low, below detection limit in most of the samples and with median values of 0: HCHs, including lindane, dieldrin, aldrin, opDDE. pp'DDE concentration was much higher and detectable in almost all samples and showed similar trends as PCB data, the main conclusion being again the increasing concentrations during the winter, but to a lower extend than for the PCBs (Table 4, Fig. 9).

The total mercury (ΣHg) load concerned mainly organic Hg (methyl Hg): the relative MeHg concentration, expressed as % MeHg, varied between 80 and 85%, without any detectable trend. The main observation concerns again the increase during winter, with MeHg concentrations varying in liver from 2.3 µg/ g dw in November - December to 7 in March, from 0.8 to 2.3 in muscle and from 1.6 to 6 in kidney (all $p < 0.01$) (Table 5, Fig. 10). In the period 1970/ 1981, we recorded similar ΣHg concentrations of 7.8 in liver and 6.2 in kidney (Delbeke *et al.*, 1984). Summer values were 2 in liver, 0.4 to 1 in muscle, and 1.5 in kidney in northwest Scotland (Stewart *et al.*, 1994) and northern Norway (Wenzel and Gabrielsen, 1995): the Hg concentrations obtained during winter presented here are much higher than the ones measured in summer.

TABLE 3

PCB concentration in different tissues of the common guillemot, in the different periods, expressed as sum congeners on the bases of dry and lipid weights (median values; n = number of samples).

	Σ congeners								
	µg/g dw						µg/ g lw		
period	liver	*n*	muscle	*n*	kidney*	*n*	liver	muscle	kidney
Nov. - Dec.	2.9	*18*	1.1	*18*	0.6	*8*	34.2	27.9	4.0
January	4.2	*53*	1.6	*53*	2.8	*33*	40.1	46.9	22.6
February	2.8	*45*	1.5	*45*	2.7	*39*	22.7	44.0	23.6
March	8.0	*14*	4.0	*14*	1.9	*8*	81.6	75.8	24.2

* *kidneys were not collected during the 2 first years of this study.*

TABLE 4

o,p'DDE concentration in different tissues of the common guillemot (see table 3).

	DDE								
	µg /g dw						µg/ g lw		
period	liver	*n*	muscle	*n*	kidney	*n*	liver	muscle	kidney
Nov.-Dec.	0.4	*18*	0.2	*18*	0.1	*8*	3.4	5.8	0.8
January	0.8	*53*	0.3	*53*	0.4	*33*	7.9	8.6	3.8
February	0.4	*45*	0.3	*45*	0.5	*39*	4.4	9.8	3.8
March	1.3	*14*	0.5	*14*	0.4	*8*	14.3	8.9	3.5

Our winter data were compared with summer values obtained in Scotland, since most of the guillemots wintering in the southern North Sea seem to belong to the scottish breeding populations (out of a total of 27 recoveries of ringed guillemots along the Belgian coast, 17 originated from Scotland, and few from Germany, Sweden and England: W Roggeman, pers. comm.). This is why we propose a kind of yearly cycle of Hg levels in guillemot, by integrating our winter values and the summer values of Stewart *et al.* (1994)(Table 6, Fig. 11). These data reflect important fluctuations of the Hg contamination with time, with liver and kidney levels showing differences of 1 order of magnitude and more, while muscle values varied by a factor 6. As a consequence, liver to muscle ratios also vary, and are much higher in winter. This observation is entirely consistent with the interpretation that contamination is recent and actually increases during winter, considering that liver and kidney react much faster to changes of contamination than muscle.

Mercury concentration was determined in other species as well, in liver and muscle (Table 7); some differences were noted, reflecting both differences in diet and/ or in phylogeny. Razorbill *Alca torda,* another alcid, showed concentrations very close to the ones of guillemot.

TABLE 5

Total and organic mercury in different tissues of common guillemots (see table 3).

period	total Hg liver	n	muscle	n	kidney	n	organic (Me) Hg liver	n	muscle	n	kidney	n
Nov.-Dec.	2.81	*19*	1.06	*19*	2.92	*6*	2.33	*18*	0.81	*18*	1.60	*5*
January	5.39	*57*	1.60	*58*	4.16	*35*	4.17	*44*	1.26	*44*	3.41	*18*
February	5.71	*66*	2.13	*70*	3.58	*41*	4.03	*62*	1.61	*60*	2.62	*29*
March	8.91	*14*	2.94	*16*	7.88	*8*	7.23	*14*	2.25	*14*	6.10	*3*

TABLE 6

Total mercury in different tissues of common guillemots collected in different periods.

period	*n*	ΣHg (µg/g dw) liver	kidney	muscle	ratio liver-muscle	*ref.* *
April	*30*	3.4	3.8	1.7	2.0	*a*
June	*27*	2.3	2.4	0.8	2.9	*a*
November	*25*	0.9	0.9	0.5	1.8	*a*
Nov. - December	*19*	2.9	2.8	1.1	2.6	*b*
January	*58*	5.7	4.5	1.9	3.0	*b*
February	*70*	6.2	3.8	2.3	2.7	*b*
March	*16*	9.4	9.9	3.1	3.0	*b*

* *a: Stewart et al., 1994 (mean); b: this study (median).*

TABLE 7

Total and organic Hg in other seabird species, beached on the Belgian coast during winter (µg/ g dw, see table 3).

species	ΣHg liver	*n*	muscle	*n*	kidney	*n*	organic (Me)Hg liver	*n*	muscle	*n*	kidney	*n*
Fulmarus glacialis	5.0	*12*	1.3	*12*			3.3	*11*	1.1	*12*		
Sula bassana	4.6	*4*	1.9	*4*			3.6	*4*	1.9	*4*		
Melanitta nigra	8.4	*17*	2.1	*17*			3.5	*13*	0.9	13		
Rissa tridactyla	2.9	*9*	1.6	*9*			2.1	*6*	1.0	6		
Larus argentatus	3.0	*13*	0.9	*13*	1.6	*7*	2.0	*13*	0.8	*13*	1.1	*7*
Larus ridibundus	2.4	*24*	0.8	*23*	1.5	*10*	1.7	*19*	0.7	*18*	1.2	*7*
Alca torda	11.1	*24*	4.1	*23*			9.5	*22*	4.1	*20*		

Conclusion

Organochlorines concentration in guillemots is higher in winter than summer and increases during winter in the southern North Sea. Hg concentration also varies as a function of time, decreasing during summer in North Atlantic water ecosystems, and increasing during winter in the southern North Sea. This cycle can be explained by a simple uptake - excretion model, with a turn-over of about 1 month: changes in the contamination of food consumed in summer and winter allow to entirely explain the observed variations. Other factors such as excretion through moult, or eggs laying, seem to play a limited role only, if any.

These results on organochlorines and Hg concentrations clearly must be normalised for the collecting date of the birds before allowing any comparison for detecting temporal or geographical differences. Comparing means or medians without such a normalisation could lead to wrong conclusions such as variations from year to year, while the main factor influencing these figures is the collection date: years with an early winter mortality of guillemots provide lower PCBs and Hg concentrations than years with a later mortality.

When such data are to be used in a monitoring approach, the low values obtained in late autumn could reflect the contamination of North Atlantic water ecosystem, and the high values in early spring the one of southern North Sea ecosystem. These last values, with medians of 8 ppm dw $\sum$congeners (corresponding to almost 30 ppm "total" PCBs) and 9 ppm dw Hg in liver, could be toxic and could have caused cachexy and/or gastro-enteritis, the main pathological observations among guillemots beached in the southern North Sea (Jauniaux *et al.,* 1997).

This study was partially funded by the Impulse Programme in Marine Sciences, the Prime Minister's Services - Office for Scientific, Technical and Cultural Affairs (MS/03/31) and the European Commission (13M/91/138, 1366-188901). Collection of samples was possible thanks to P Meire, J Seys, H Offringa, J Tavernier and the rehabilitation centres of Ostend, Nieuwpoort, Blankenberge and the Zwin. Weight, oiling and pathology data were kindly provided by T Jauniaux. Data on the "other species" were part of theses by F Adrien, J Gandikote, AV Iordache and C Mihai.

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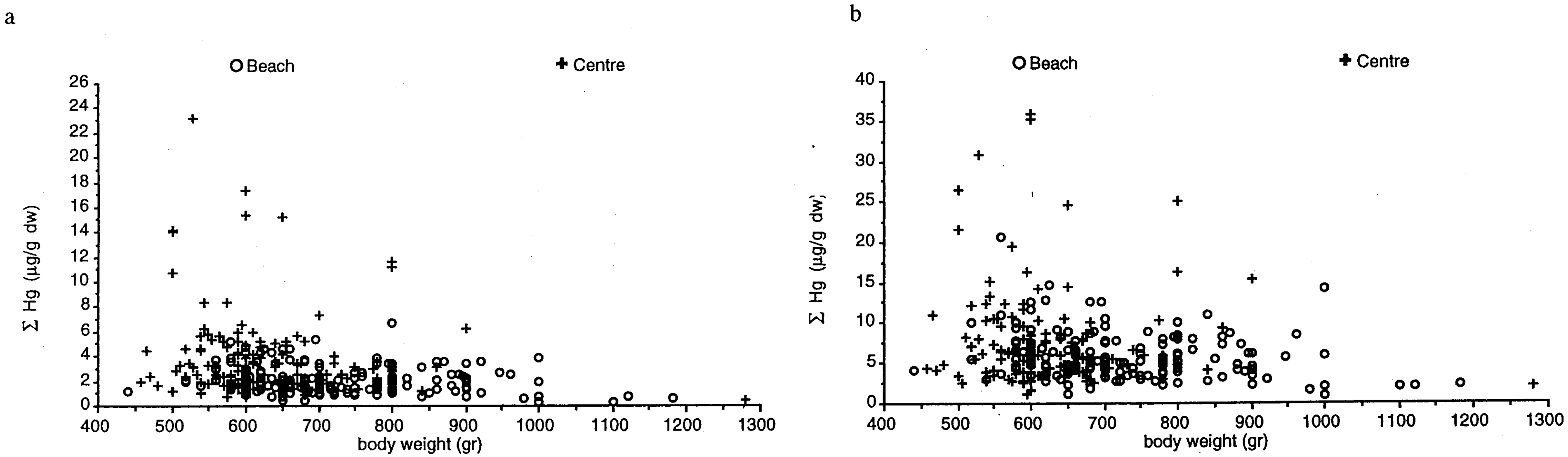

Fig. 1. Mercury concentration in common guillemots *Uria aalge* beached on the Belgian coast: total Hg in muscle (1a; n = 298) and in liver (1b; n = 278) as a function of total body weight (g). Circles: found dead on the beach; crosses: beached alive, dead in rehabilitation centres.

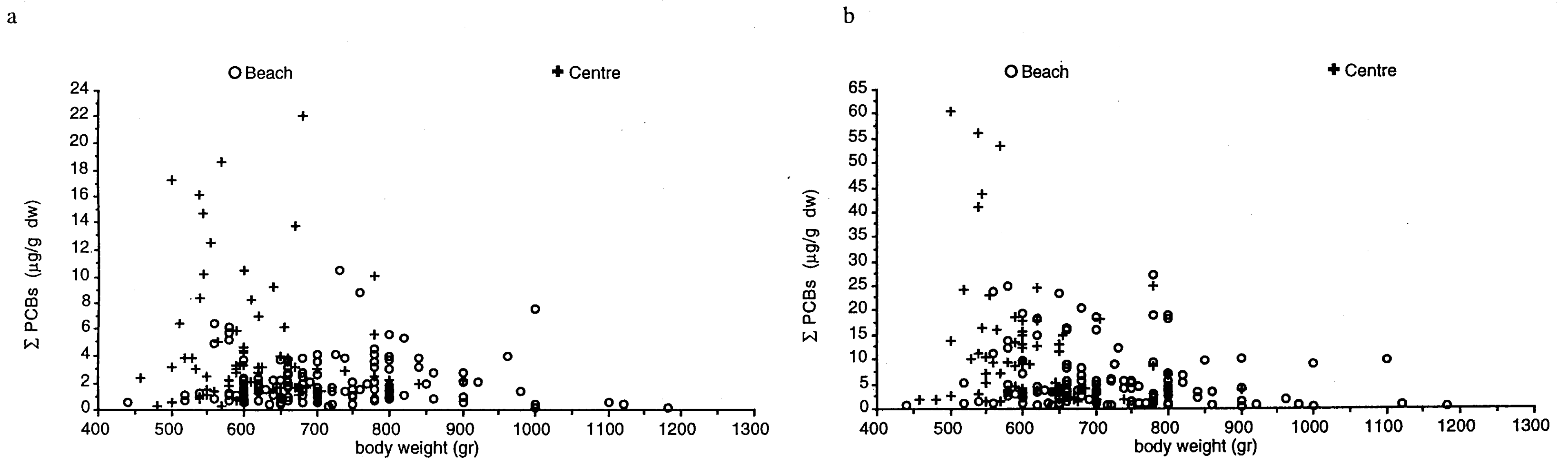

Fig. 2. PCB concentration in common guillemots: Σ congeners in muscle (a; n = 206) and in liver (b; n = 198)(µg/ g dw). See Fig. 1.

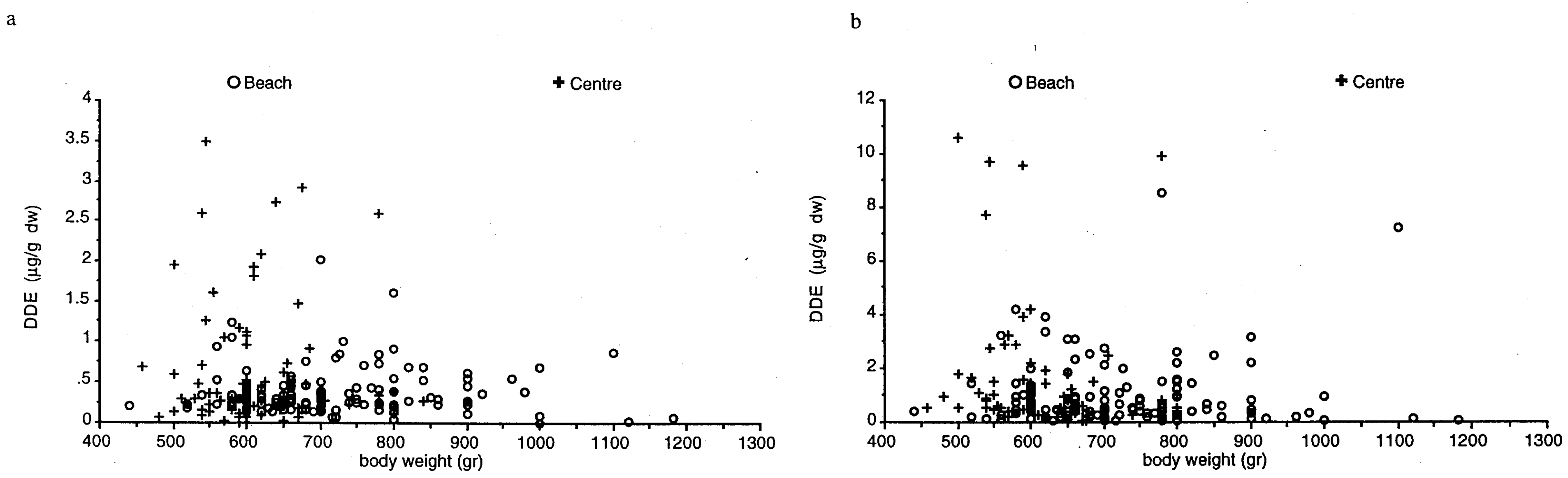

Fig. 3. Organochlorine pesticides concentration in common guillemots: opDDE in muscle (a; n=206) and in liver (b; n=198). See Fig. 1.

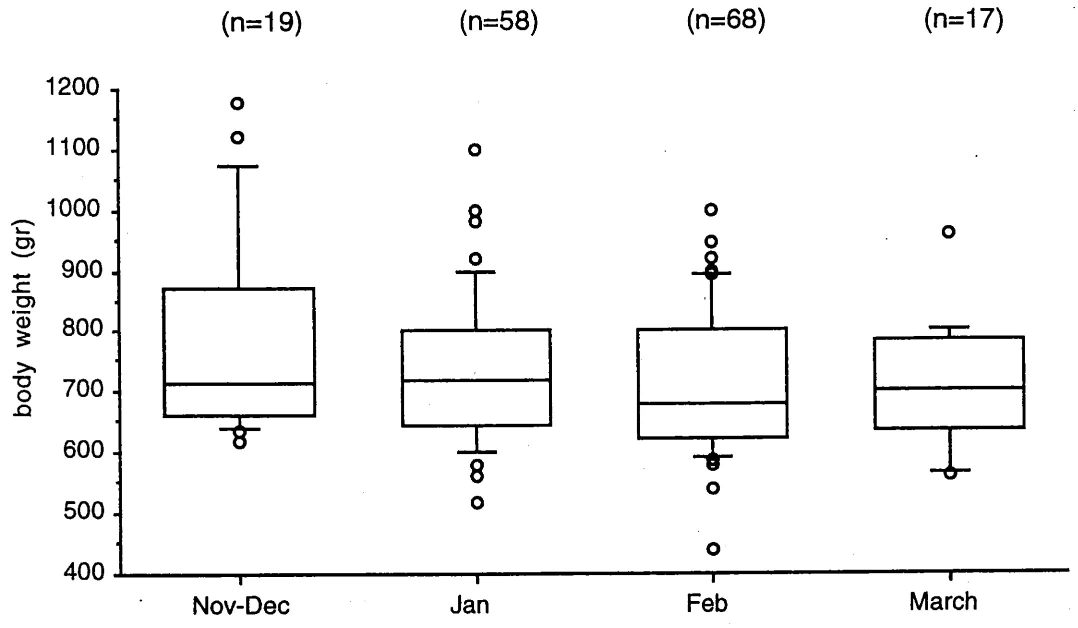

Fig. 4. Total body weight of common guillemots (g) as a function of collection date. In the boxes: median value (50%), 75%, 90%, outsiders (n = number of birds); p > 0.3.

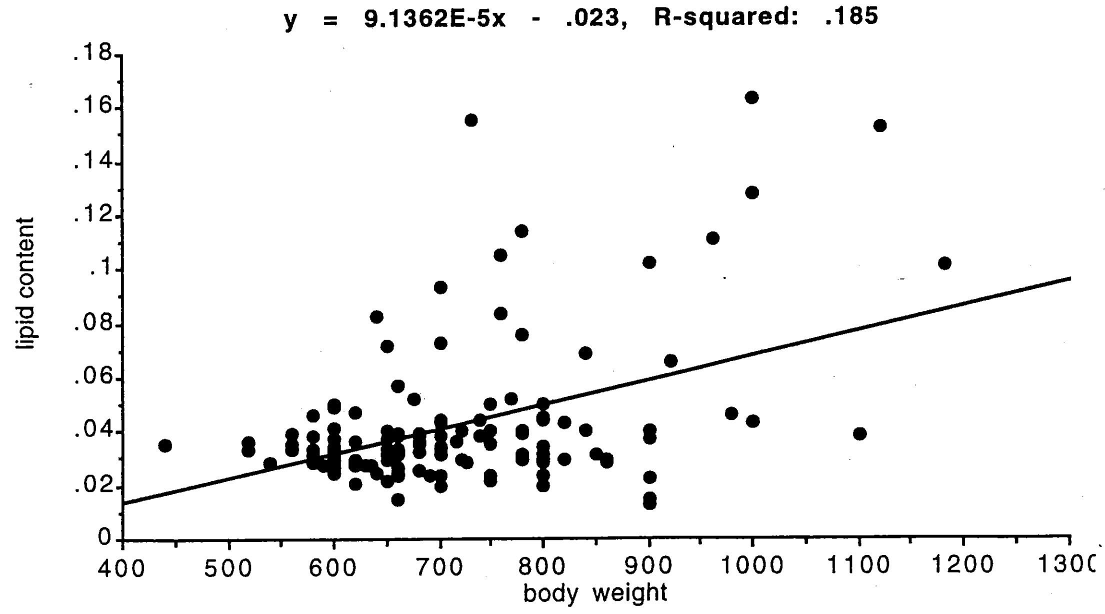

Fig. 5. Polar lipids content of the muscle of common guillemots (g /g dw) as a function of total body weight (g)(n = 206).

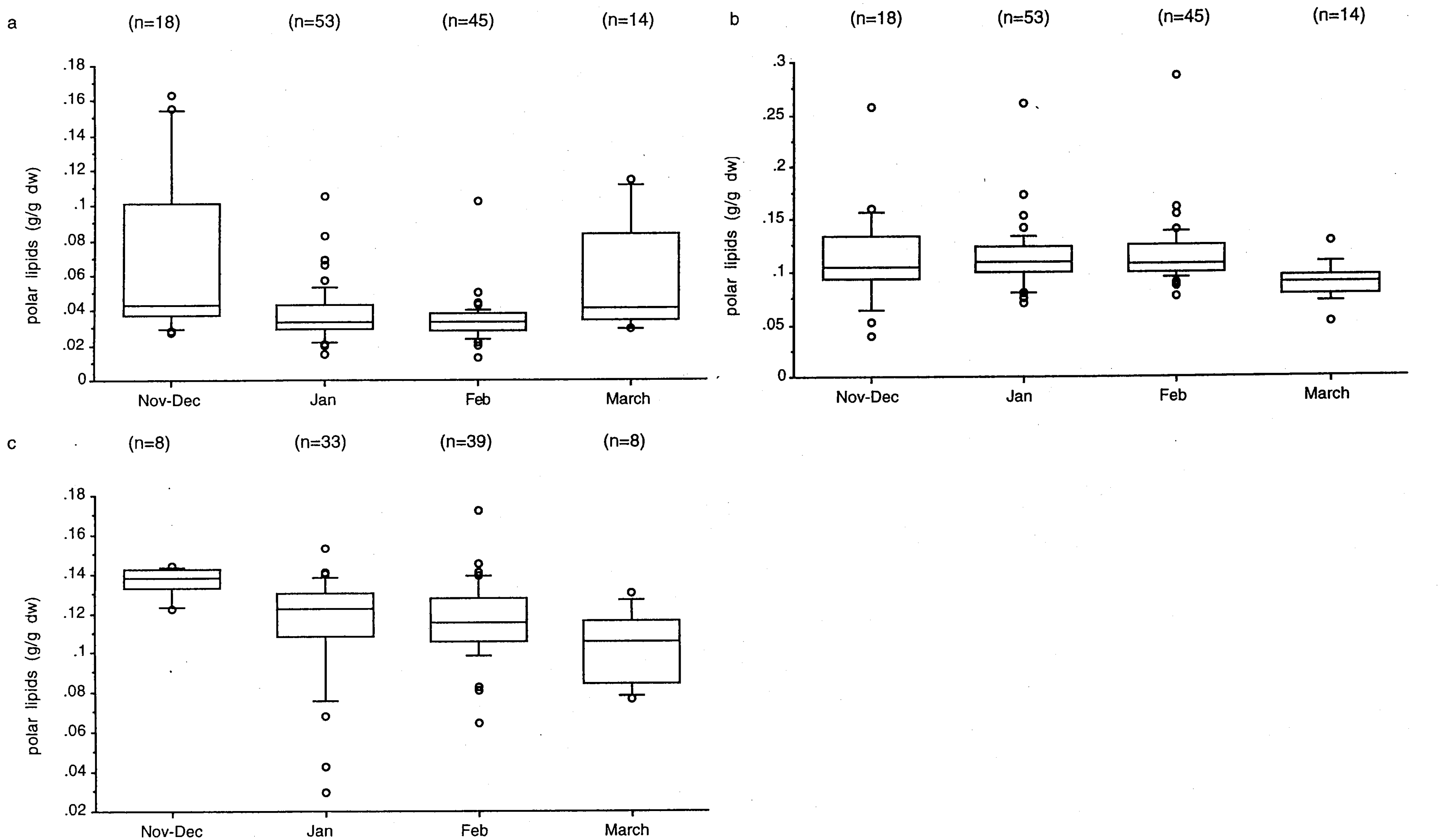

Fig. 6. Polar lipids content of common guillemots tissues as a function of collection date, in muscle (a), liver (b) and kidney (c)(g/ gdw).

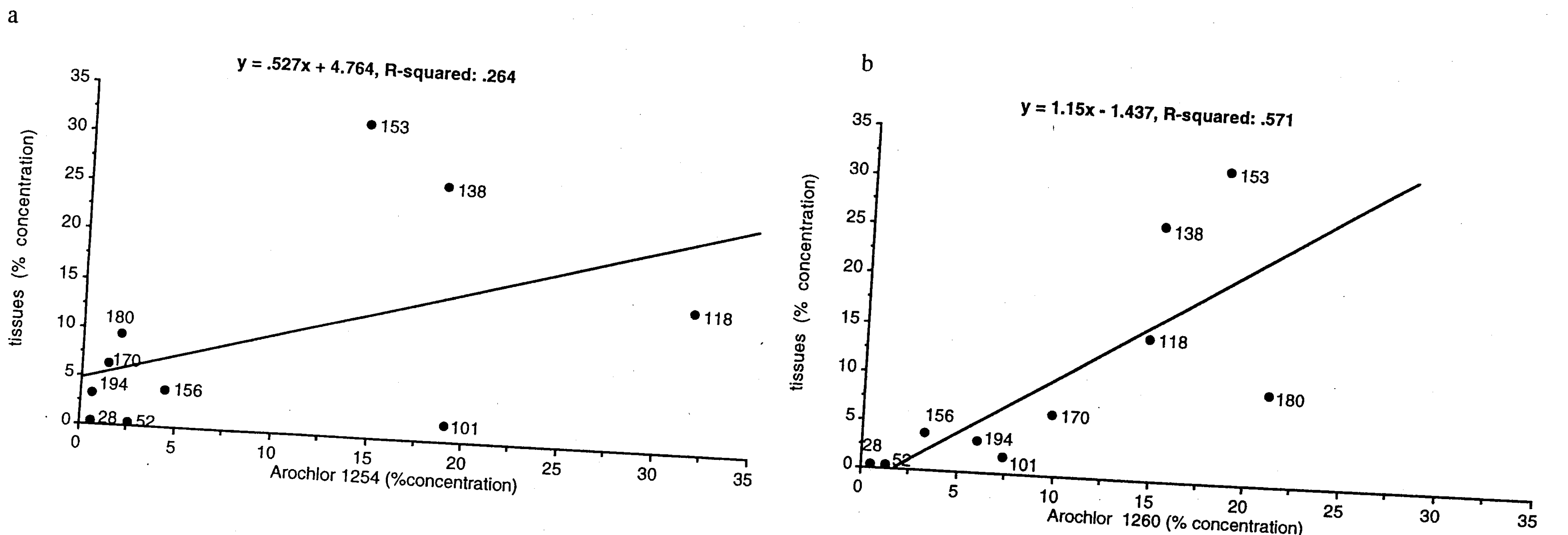

Fig. 7. Correlation between the relative concentration of congeners (as % of Σ congeners) in tissues of common guillemot and in the standard mixtures Aroclor 1254 (a) and 1260 (b)(median values; 348 tissues, 13 Arochlor 1254 and 20 Aroclor 1260).

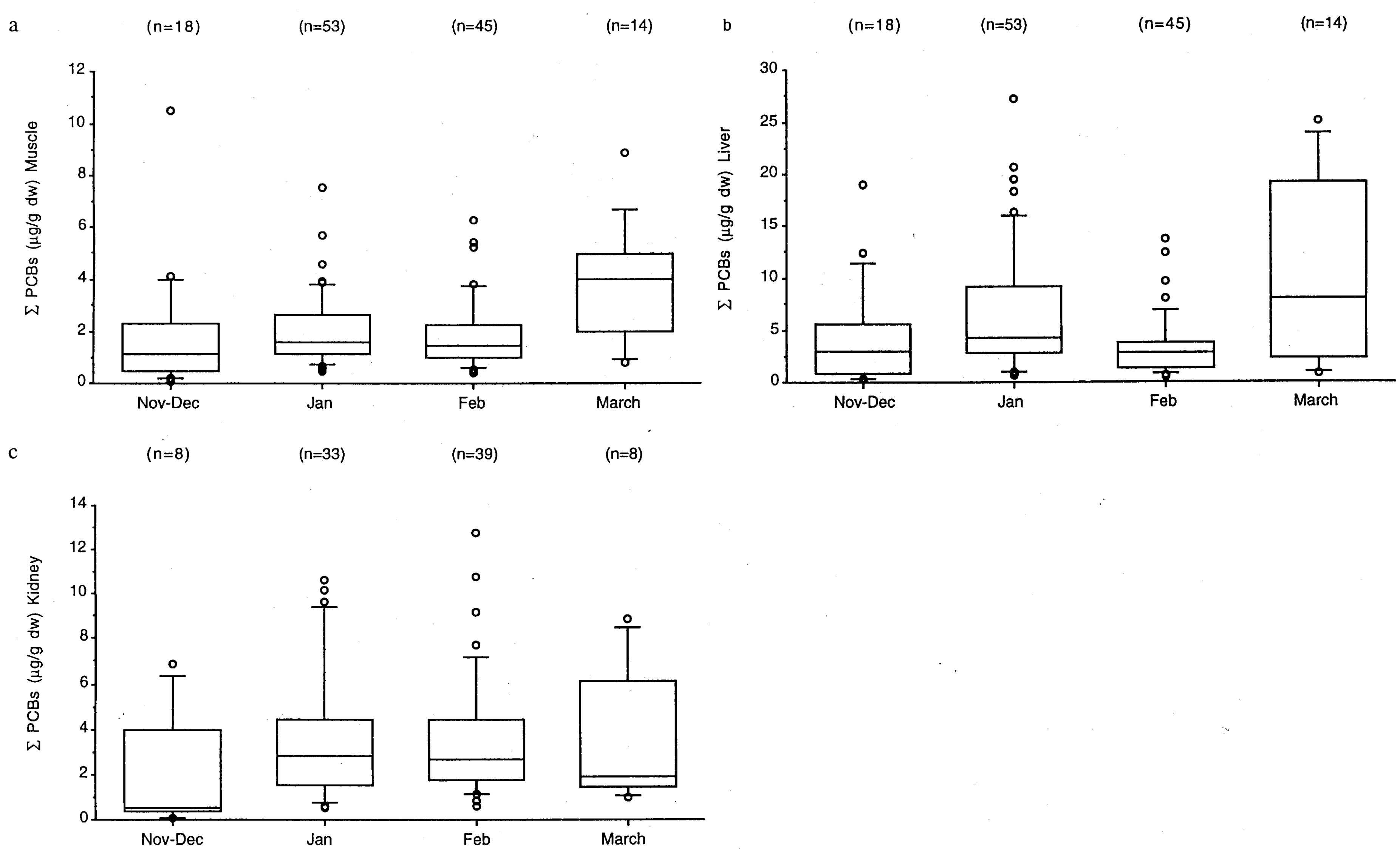

Fig. 8. PCB concentration in common guillemot: Σ congeners as a function of collection date, in muscle (a), liver (b) and kidney (c)(µg/ g dw).

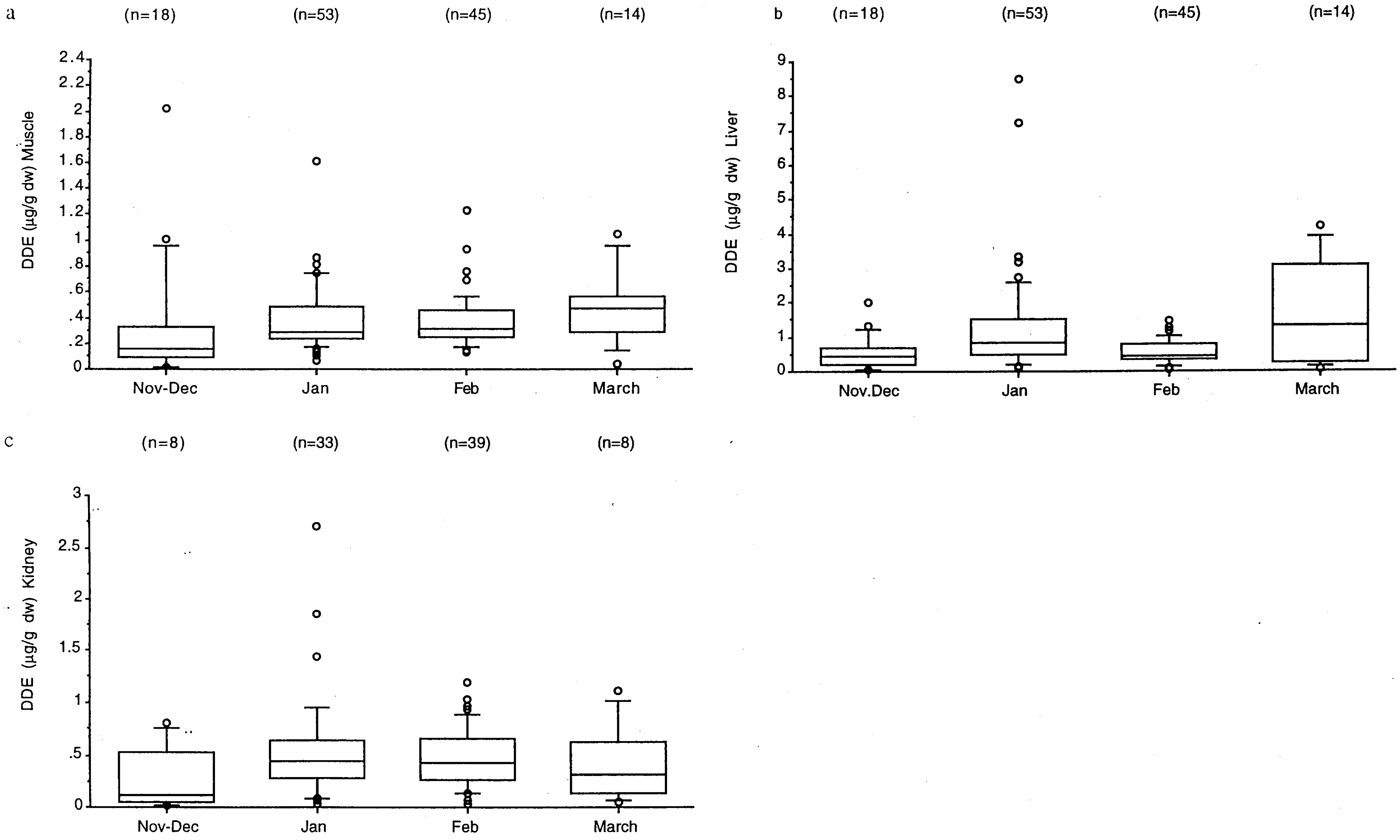

Fig. 9. Organochlorine pesticides in common guillemot as a function of collection date: opDDE in muscle (a), liver (b) and kidney (c)(µg/ g dw).

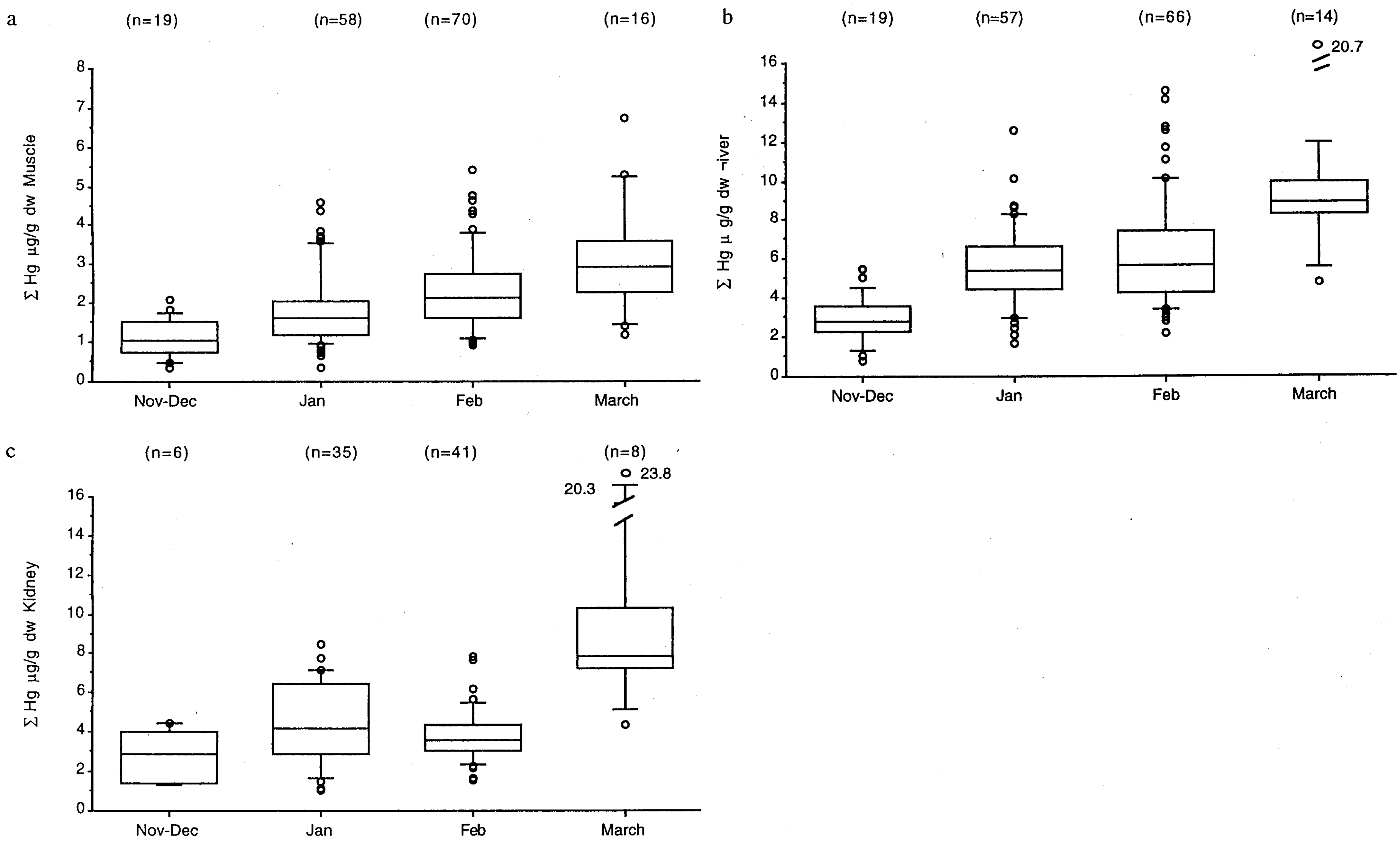

Fig. 10. Mercury in common guillemot as a function of collection date: ΣHg in muscle (a), liver (b) and kidney (c)(µg/ g dw).

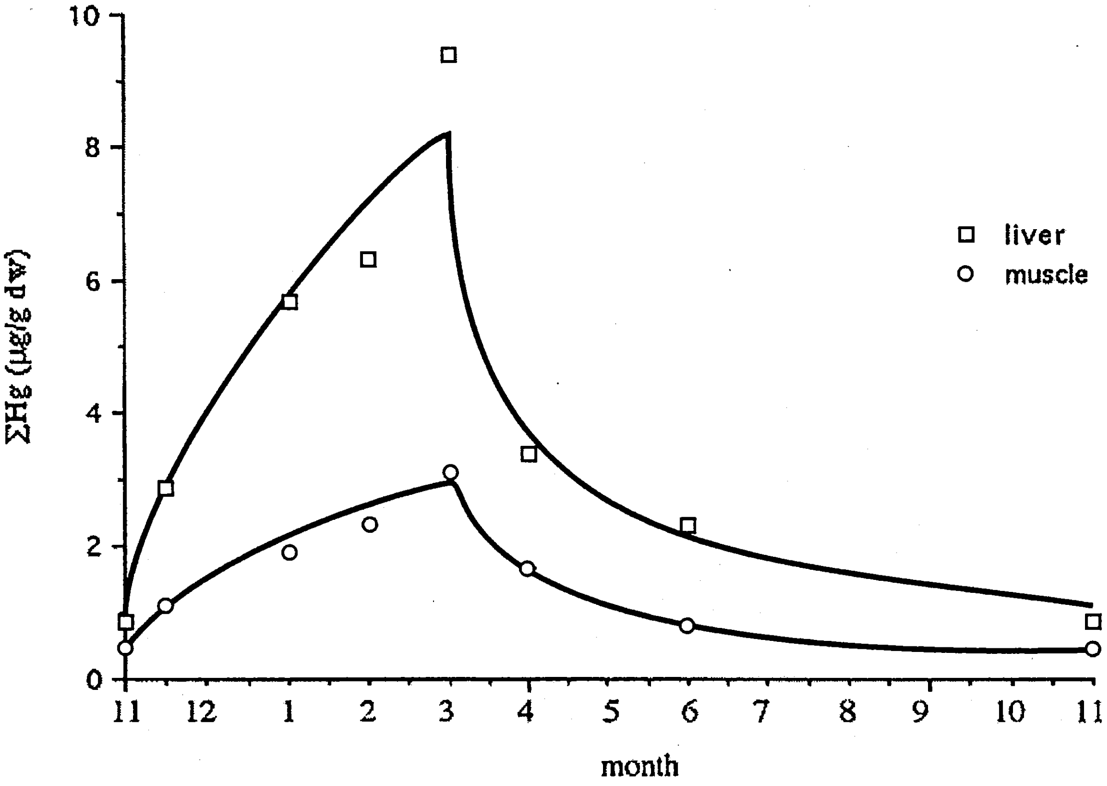

Fig. 11. Annual cycle of Hg concentration in liver and muscle of the common guillemot. April - November: Stewart *et al.*, 1994; mid - November - March: this study. Curve fitted by eye.

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Annex 3

On the stranding of four sperm whales on the Belgian coast, November 18, 1994. Preliminary scientific report, Laboratory for Ecotoxicology VUB

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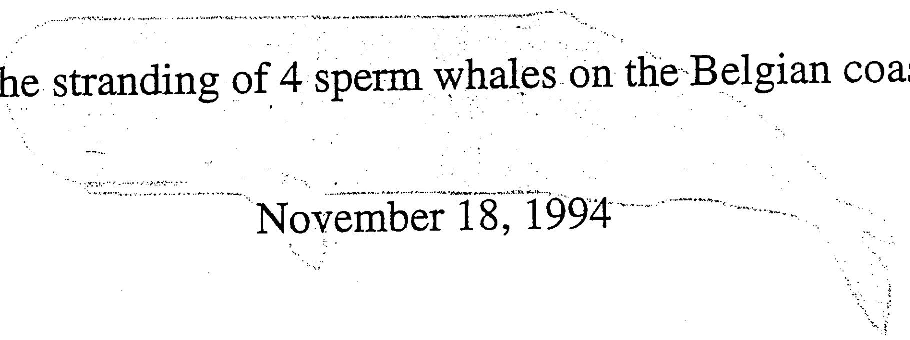

On the stranding of 4 sperm whales on the Belgian coast, November 18, 1994

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

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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.

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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3.2. Administrative and technical coordinations

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ROGGEMAN W. [5]	Head technical team
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(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.

<u>Weather conditions:</u> 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) x 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) x 1.14 = 21,940 kgs (estimate)

Sperm whale 4 :

(34,040 + 165) x 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.

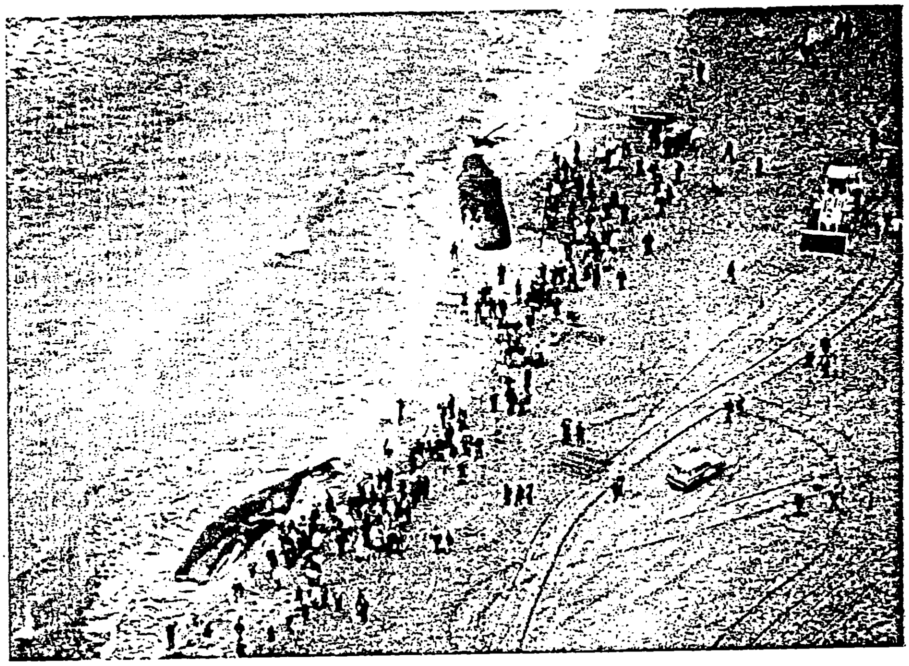

Figure 1 : Sperm whales #1 (lower left) and #2 on the beach of Koksijde, Nov. 18, 1994 (photograph E. Donnay, MUMM).

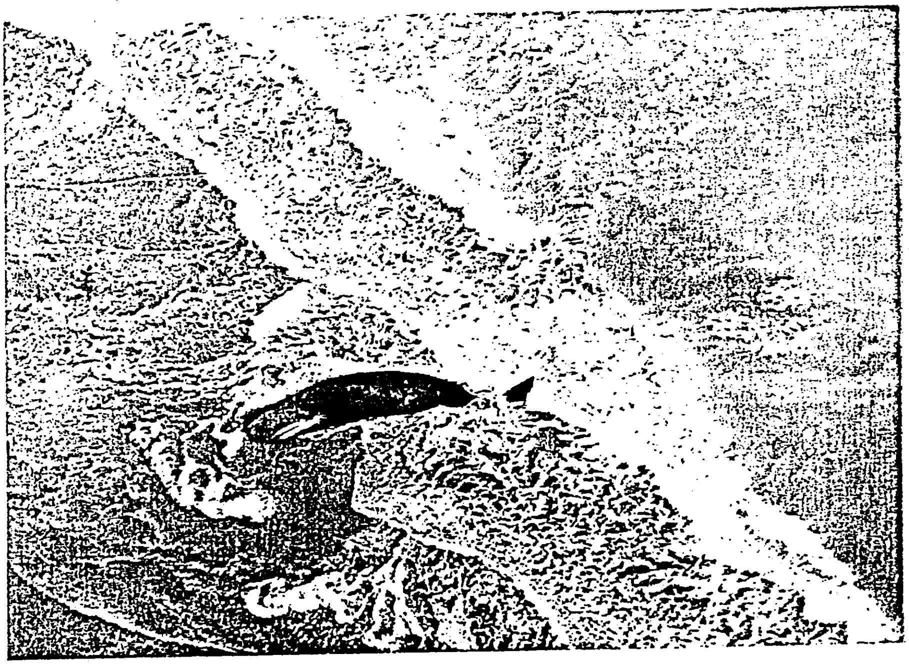

Figure 2 : Sperm whale #3 on the beach of Koksijde, Nov. 18, 1994 (photograph E. Donnay, MUMM).

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).

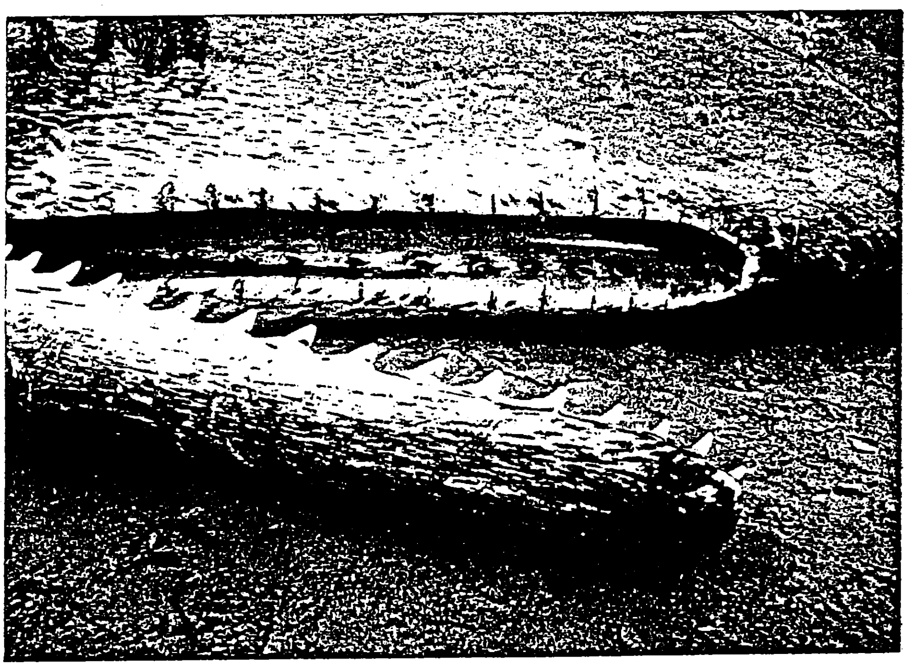

Figure 4 : Sperm whale #2. Post mortem wounds of the upper lip due to rythmic closure of the lower jaw, Nov. 18, 1994 (Photograph E. Donnay, MUMM).

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	1	90	184	84
	2	100	237	140
	3	95	140	
	4		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				
Total content (mgCd/kg fw)	32	21	22	12
I.F.	19.5 (61%)	9.5 (45%)	10.3 (47%)	1 (8%)
S.F.	12.5 (39%)	11.5 (55%)	11.7 (53%)	11 (92%)
MT	1.9 (6%)	2.1 (10%)	2.6 (12%)	11 (92%)
Kidney				
Total content (mgCd/kg fw)	83	101		
I.F.	15.8 (19%)	15.2 (15%)		
S.F.	67.2 (81%)	85.8 (85%)		
MT	21.6 (26%)	54.9 (54%)		

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

Table 6: 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. S.F. MT	85% 15% 2%	95% 5% <1%	84% 16% <1%
Kidney			
Total content (mgHg/kg fw)	0.5	0.6	
MeHg	21%	21%	
I.F. S.F. MT	72% 28% 1 %	70% 30% 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)

Proceedings symposium "North Sea Sperm Whales, one year after", Koksijde, November 1995.

Annex 4

Mercury and organochlorines in four sperm whales stranded on the Belgian coast, November 1994.

Joiris C., L. Holsbeek, G. Tapia and M. Bossicart, in press.

not to be cited

Mercury and organochlorines in four sperm whales stranded on the Belgian coast, November 1994.

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Abstract

Four sperm whales (3 sub adult males stranded on the Belgian coast, a 4th older male found dead at sea) were analysed for total mercury, methylmercury and organochlorines. All 4 were part of a total of 24 sperm whales which stranded on the North Sea coasts over a period of 6 months, a highly unusual phenomenon. Total mercury levels ranged from 0.5 μg/g fw in kidney and 1 in muscle up to 15 in liver. The finding of at least 90% of the mercury in its inorganic form, confirms the existence of detoxification mechanisms in the liver of cetaceans. Expressed on a lipid weight basis, PCB concentrations in muscle, liver, kidney and blubber ranged from 10 to 25 μg/g. These results are in the same order of magnitude of literature data, what however does not imply that there is no impact on the populations. Social behaviour rather than a direct effect of pollutants caused the stranding of the 3 younger animals: they apparently remained close to the older one (the leader?) dead at sea, and stranded in very shallow water.

The fact that no other cases of mass mortality were reported out of the North Sea suggests no immediate threat to the whole North Atlantic population. The finding, however, of at least 3 more sperm whales dead at sea during the 1994/95 winter period, points towards a non-natural phenomenon. Indirect impact of anthropogenic pollutants influencing the behaviour and/or the health of a social cluster is only one of the possible hypothesis to explain why a large number of sperm whales got trapped in the North Sea.

Introduction

In the early morning of November 18th, 1994, 3 sperm whales *Physeter macrocephalus* were found stranded side to side on the western part of the Belgian coast near the town of Koksijde. All three were young males between 14.5 and 15.5 m in length, corresponding to an estimated age of 20 to 30 years (Martin, 1980). The same day, a fourth and larger animal (nr. 4, 18m, estimated age 55+ years) was found dead at sea not far from the coastline at the town of Nieuwpoort; he was towed to land for further study.

The general state of the older animal (#4) clearly indicated that he must have been dead at sea for at least 1 day; its state of decay did not allow a full autopsy nor ecotoxicological sampling. The other sperm whales -stranded alive but already dead when found- died during the night.

Recent strandings and mortalities in the North Sea of in total 24 sperm whales over a period of 6 months (1 UK, 1 Netherlands, 1 Germany, 3 dead at sea Norway, 4 Belgium, 11 Orkney Islands UK & 3 Netherlands) must raise some questions about the health status and the prospects of the sperm whale populations. In particular, heavy metals and organochlorine residues are at least suspect having a negative impact on higher marine trophic levels.

Analyses for total and organic mercury content as well as for PCBs and a series of organochlorine pesticides were performed on muscle, liver, kidney and blubber tissue when available (i.e. not for whale 4); a comparison of our results with complementary data for selenium, metallothioneins, cadmium, lead, copper, chromium and zinc (Oceanology, University of Liège[1]) on one hand and the results for anatomopathology (Veterinary Medicine, University of Liège[1]) on the other hand, allowed us to draw up a general conclusion about the health status of the animals involved. Finally, we tried to answer the question whether or not, levels of stable pollutants might have had any influence, directly or indirectly, on the death of the animals involved and in the mass mortality of sperm whales in the North Sea area during the winter of 1994/95.

Methods

Total mercury analysis was analysed using specific Atomic Absorption Spectrometry performed on a Perkin-Elmer MAS-50 Mercury analyser: samples were mineralised at 200°C with sulphuric acid H_2SO_4 and oxidized by adding hydrogen peroxide H_2O_2. A few drops of potassium permanganate $KMnO_4$ were added to control whether the oxidation is complete, the excess of $KMnO_4$ being removed by adding hydroxylammonium chloride $HONH_3Cl$. This solution was then transferred into a reaction vessel where mercury was reduced with stannous chloride $SnCl_2$ into volatile Hg° vapour.

Methylmercury was measured by ECD-gas chromatography using a 0.75 ID Supelco SP-1000 column on a Packard 437 chromatographer (isotherm 120°, injector 200°, detector 250°); Copper sulphate $CuSO_4$, sodium bromide NaBr solution and toluene were added to the dried homogenate samples. The solution was mixed the aqueous and non-aqueous (toluene) layers. After centrifugation, 5/6th of the toluene was removed and set aside, and the extraction was repeated on what remained. The methyl mercury was extracted from the toluene by addition of a solution of $Na_2S_2O_3$ which forms a very specific thiol-methyl mercuric complex. The mercury containing complex was drawn back into a fresh toluene phase by addition of NaBr solution.

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[1] see papers by respectively Bouquegneau and Coignoul in these proceedings.

PCBs and organochlorine pesticides concentration were determined by ECD-gas chromatography using a capillary CP-Sil 4 column on a Shimadzu 14-A chromatographer: carrier gas N2, temperature programme from 60 to 270°, injection T 270°, detector T 300° after an hexane extraction and a florisil clean-up.

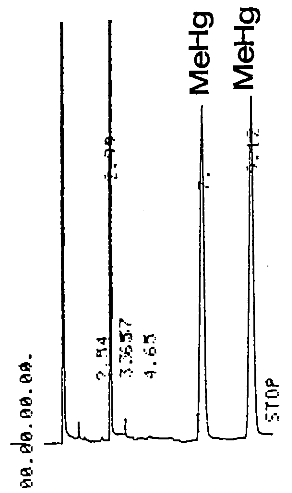

Figure 1: Chromatograph pattern for organic mercury analysis: double injection 1 µl.

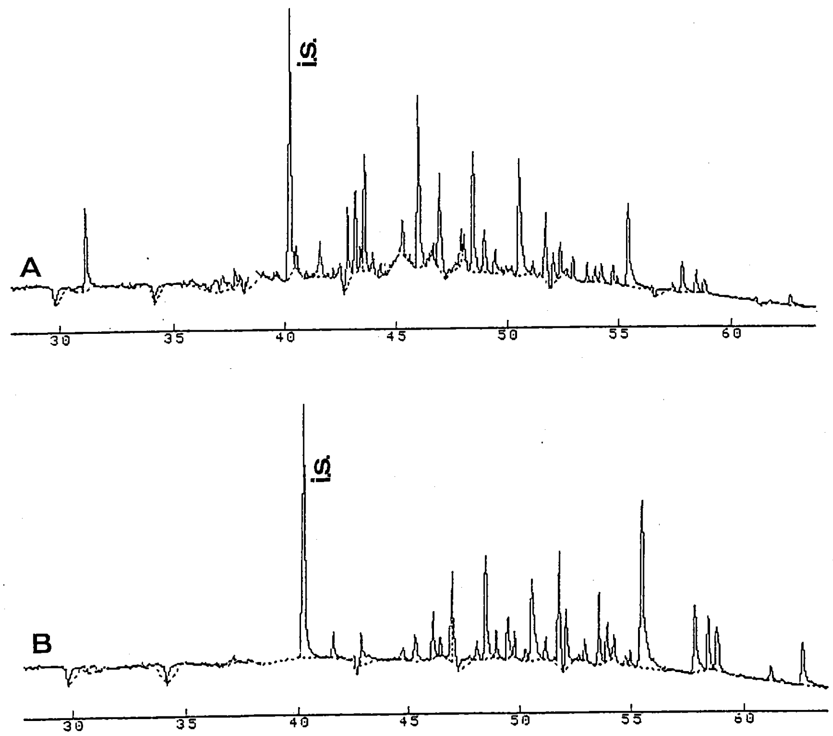

Figure 2: Chromatograph for PCB analysis: blubber sperm whale 1 (A) and reference Aroclor 1260 (600 pg/µl, B)

Results and discussion

Concentration of total mercury ranged from 0.5 μg/g fw in kidney up to 15 in liver. The organic mercury fraction (methylmercury) represented on average 6% in liver, 21% in kidney and 76% in muscle, reflecting the existence of a slow detoxification mechanism in the liver (Koeman et al., 1975; Martoja & Viale,1977; Martoja & Berry, 1980; Capelli et al., 1989; Carlini & Fabbri, 1989; Wageman et al., 1981; Hansen et al., 1990; Joiris et al, 1991; Paludan-Muller et al., 1993)

The importance of age has been frequently established where it concerns stable pollutants and marine mammals (Falconer et al., 1980; Subramanian et al., 1988; Itano & Kawai, 1981; Joiris et al, 1991); although not entirely sampled, no obvious higher concentrations were found in case of the older whale. Main differences to be expected, however, could have been at the level of the liver.

Data for total PCB concentration (calculated on the base of Aroclor 1260 standard mixture) ranged from 0.3 μg/g fw in muscle and liver up to 13 in blubber. Expressed on a lipid weight base, all but one tissues had concentrations exceeding 10 μg/g. DDE concentrations never exceeded 7 μg/g lw; DDT, Aldrin and Heptaclor were below the detection limit of 0.2 μg/g lw.

These results not only show high concentrations, they also indicate a very high total load if we consider that e.g. 30 to 40% of the body weight consists of blubber, an equal amount to be muscle. Based on this assumption the total burden for each sperm whale can be roughly estimated at 150g PCBs, 15g Hg, 20g DDE , 7g Cd and 7g Pb. This implies that each animal represents a load of at least 200g of stable pollutants. This fact has implications on the treatment of the carcasses.

A comparison with literature data is difficult to make, not only because data are not abundant, but also because different units were used. However, our data correspond with the levels mentioned in recent literature (table 1). The fact that the levels of organochlorine compounds do not exceed levels descibed in literature data does not imply that no effect could evolve from these concentrations. Comparable organochlorine levels were found to cause, or at least were suspected to have caused, severe problems (reproduction, immune-deficiency) in cetaceans and pinnipeds (Helle et al., 1976; Subramanian et al., 1987 ; Addison, 1989; Simmonds et al., 1993; Deguise et al., 1994; Tanabe t al., 1994; Kuehl and Haebler, 1995).

	n	M	L	K	Bl	unit	origin	reference
ΣHg	1	2.1			4.1	µg/g fw	France Meditt.	Thibaud & Duguy 1973
ΣHg	1	0.9	19			µg/g fw	Belgium	Joiris et al., 1991
ΣPCB	2				39.1	µg/g lw	France Atl.	Alzieu & Duguy, 1979
ΣPCB	8	24.1	30.1	9.4	9.9	µg/g lw	Spain Atl.	Aguilar, 1983
	6	30.7	18.6	9.2	15.6	µg/g lw	id.	id.
ΣPCB	10				10.5	µg/g lw	Iceland	Borrell, 1993
pp'DDE	8	2.3	4.0	3.3	2.9	µg/g lw	Spain Atl.	Aguilar, 1983
	6	2.9	6.5	2.9	4.0	µg/g lw	id.	id.
pp'DDE	10				4.2	µg/g lw	Iceland	Borrell, 1993
pp'DDE	6				3.6	µg/g fw	California	Wolman & Wilson, 1970
pp'DDE	12				0.22	µg/g fw	South Africa	Henry & Best, 1983

Table 1: Selected literature data on Hg, PCBs and DDE concentration in sperm whales muscle (M), LIver (L), kidney (K) and blubber (Bl) (n: number of samples).

An overall comparison with the results for other heavy metals showed elevated levels for PCBs and non-detoxified cadmium, while on the other hand mercury was detoxified and bound to selenium (Bouquegneau et al., same proceedings). All 4 sperm whales were concluded to be in poor health conditions.

Conclusions

It cannot be excluded, but it seems unlikely that the recent strandings and mortalities in the North Sea of in total 24 sperm whales over a period of 6 months were purely accidental. A comparison of the results obtained by the 3 participating teams on pathology and toxicology made it clear that social behaviour, more specifically the fact that the 3 younger bulls must have stayed with the dead older one, leading to their own stranding and death after being trapped in shallow water by tidal movements.

Elevated concentrations for Hg, PCBs or Cd, are most probably not the direct reason for the stranding or death of the four Belgian sperm whales. On a larger, North Sea scale, it is as unlikely that pollution would be the direct cause for the stranding or death of 24 sperm whales. If the North Atlantic sperm whale population was affected directly by actual levels of stable pollutants, other mortalities on other locations should have confirmed this hypothesis. This is clearly not the case.

Nevertheless, this does not imply that contaminant levels could not have indirectly affect the behaviour and/or the health of some of the members of a larger social group and so might also have caused the stranding of the rest of its members. This hypothesis also implies that all 4 were member of the same social group, probably even part of a greater bachelors group. It seems indeed highly unlikely that within the same year, several sperm whales would die of old age - at sea - in the North Sea area.

The fact that all 4 sperm whales were in poor health condition: lesions, ulcers, low body weight and high concentrations of stable pollutants cannot be neglected. The most interesting question about the mortality at sea of the older individual, however, was not (yet) revealed, due to incomplete sampling.

Acknowledgments
This study was conducted as part of the contract on 'Pathology and Ecotoxicology of seabirds and marine mammals in the North Sea and adjacent areas', funded by the Belgian Federal Services for Scientific, Technical and Cultural Affairs.

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