

**BELGIUM PROGRESS REPORT ON CETACEAN RESEARCH MAY 2004 TO APRIL 2005, WITH
STATISTICAL DATA FOR THE CALENDAR YEAR 2004**

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1. Species and stock studied

| Common name | Scientific name | Area/stock | Items referred to |
|---------------------------|-----------------------------------|------------|--|
| Sperm whale | <i>Physeter macrocephalus</i> | North Sea | 2.1.1, 6.2.1, 6.3 |
| Common minke whale | <i>Balaenoptera acutorostrata</i> | North Sea | 6.2, 6.2.3 |
| Harbour porpoise | <i>Phocoena phocoena</i> | North Sea | 2.1.1, 7.1, 7.2, 9.1, 9.2, 9.3, 9.4, 9.5 |
| White-beaked dolphin | <i>Lagenorhynchus albirostris</i> | North Sea | 2.1.1, 9.1 |
| Common bottlenose dolphin | <i>Tursiops truncatus</i> | North Sea | 2.1.1 |

2. Sightings data

2.1 Field work

2.1.1 SYSTEMATIC

Ship-based seabird and marine mammal surveys along the Belgian coastline are organized regularly and observations are recorded in the MUMM (Management Unit of the North Sea Mathematical Models), database¹. Total number of records in the MUMM database in 2004 for Belgian waters is 89. Species observed were harbour porpoises *Phocoena phocoena* n= 51, common bottlenose dolphin *Tursiops truncatus* n= 30, white-beaked dolphins *Lagenorhynchus albirostris* n= 7 and sperm whale *Physeter macrocephalus* n= 1

2.1.2 OPPORTUNISTIC, PLATFORMS OF OPPORTUNITY

For 2004, no platform of opportunity was used for Belgian waters.

2.2 Analyses/development of techniques

In 2005, two platforms of opportunity have been used or will be used. Firstly, on the Thalassa (oceanographic research vessel of Ifremer Institute, France), cruising in the central and southern North Sea in February. Secondly, on the Belgica, Belgian oceanographic vessel, from Cadix (Spain) to Zeebrugge (Belgium) via Cork (Ireland), in May and June.

3. Marking data

None.

4. Tissue/biological samples collected

4.1. Samples from alive or from directed catches

None.

4.2. Samples of stranded or by-caught animals

¹ http://www.mumm.ac.be/EN/Management/Nature/search_strandings.php

Following body conservation codes, samples are collected for histopathology, toxicology and microbiology (Jauniaux *et al.*, 2002b). For histopathology, samples of eye, skin, liver, lymph nodes, gonad, reproductive tract, oesophagus, stomach, intestine, kidney, urinary bladder, pancreas, lung, heart, thyroid, thymus and brain, and all tissues with lesions are fixed in 10% buffered formalin. For toxicology, blubber, muscle, liver, kidney and sera are frozen (-20°C) and for microbiology, lesions are directly analyzed or frozen (-20°C). In 2004, 41 cetaceans were completely sampled.

4.3. Analyses/development of technique

Stable carbon and nitrogen analyses ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) are also performed in large cetaceans from the Mediterranean Sea in order to trace their diet (collaboration with Cristiana Miglio and Prof. Renzo Capelli, University of Genova DICTFA (Dipartimento di Chimica e Tecnologie Farmaceutiche ed Alimentari)).

5. Pollution studies

Organic and metal contaminant (mercury, methylmercury, cadmium, zinc, copper, iron and selenium) analyses are systematically performed on available samples (blubber, liver, kidney, muscle) in the same way as for small cetaceans (items 9.3)

6. Statistics for large cetaceans

6.1 Direct catches (commercial, aboriginal and scientific permit)

None.

6.2 Other non-natural mortality for the calendar year 2004

| Species | Area/stock | Males | Females | Total | Cause | Methodology |
|--------------------|------------|-------|---------|-------|--------------|-------------|
| Common minke whale | North Sea | 0 | 1 | 1 | entanglement | postmortem |

A common minke whale (*B. acutorostrata*) was found dead at sea during bird observation program on December 14, 2004. The whale was brought back and forwarded to the Department of Pathology (Veterinary college, University of Liege) to be necropsied, following standard procedure (Jauniaux *et al.*, 2002b). Based on post-mortem observations, it was diagnosed that the minke whale was dead following entanglement in fishing net.

6.2.1 STRANDINGS OR DEAD WHALES ENCOUNTERED AT SEA

| Species | Sex | Location | Cause of death | Det. | Source of contact institution |
|-------------|-----|---------------------|----------------|------|--|
| Sperm whale | M | 51°08'N, 02°39'E | U | N | Org: Dept of Pathology Address: Veterinary college, University of Liege, B43, 4000 Liege, Belgium Contact: Thierry Jauniaux, Tel: +32 4 3664075 Email: T.Jauniaux@ulg.ac |

6.2.2 OBSERVED OR REPORTED SHIP STRIKES

None.

6.2.3 FISHERY BYCATCH

Yes (above described common minke whale).

6.3 Earlier year s' statistics

Data concerning large cetaceans were published: for sperm whales: Jauniaux *et al.*, 1998; and for fin whales: Jauniaux *et al.*, 2000. No observation data have been published.

7. Statistics for small cetaceans

7.1 For the calendar year 2004

| Species | Area/Stock | Direct catch | Incidental mortality | | Live-captured |
|------------------|--------------------|--------------|----------------------|---------------------|---------------|
| | | reported | reported | source | Reported |
| Harbour porpoise | Southern North Sea | 0 | 7 3-10 | Gillnet* unknown | 0 |

*: GN

7.2 Earlier years' statistics

Data concerning observations and causes of death of harbour porpoises (1990-2000 period) have been published (Haelters *et al.*, 2000, 2004; Jauniaux *et al.*, 2002 a)

8. Strandings

MUMM (Jan Haelters)

9. Other studies and analyses

9.1. Pathology and microbiology of cetaceans and pinnipeds stranded along the Belgian coastline

Coordination: Dr. T. Jauniaux

The aim of the monitoring programme is to identify the lesions and the causes of death of marine mammals stranded along the Belgian coastline and to collect data and samples for collateral research programmes (see below: 9.2, 9.3 and 9.5). Biological data considered are date and place of stranding, body length and weight. Necropsy and samples (histology, virology, bacteriology, mycology) are necessary to clearly identify the causes of death (infectious diseases, net entrapment, ship collision, ...). Samples are also collected for toxicological analyses (heavy metals, organochlorines) allowing further understanding on the relationship between the health status, biological data and contaminants levels in marine top predators (see 9.2). For 2004, following small cetacean species, stranded on the Belgian coastline were necropsied: harbour porpoise and white-beaked dolphin.

9.2. Viability of the North-East Atlantic harbour porpoise and seal population (Pollution and biomarker study, contract EV/XX/806).

Coordination: Pr Jean-Marie Bouquegneau (contact: K. Das).

This study aims to investigate:

- The ecological status of the harbour porpoise in the Northeast Atlantic including the North Sea, in particular population structure and health status.
- The environmental impact at the population, individual, cellular and subcellular levels using biomarkers in both field studies and laboratory assays.

Three approaches will be considered to reach the objectives:

A. Population level: age structure and population dynamics (sex, length...).

B. Individual level will consider health status and will identify the causes of death (infectious diseases, entanglement, others).

C. Cellular and subcellular levels that will consider potential biomarkers of pollutant exposure (CALUX, cytokine expression and thyroid lesions). Chemical agents potentially responsible for the above mentioned effects are analysed in selected individuals and tissues (liver, kidney, muscle and blubber) due to their well-known negative impacts on health.

9.3. Viability of the North-East Atlantic harbour porpoise and seal population (Genetic and ecological studies, Contract number: EV/I2/46)

Coordination: Pr Jean-Marie Bouquegneau (contact: K. Das).

This project aims to assess the viability of the harbour porpoise and harbour seal populations in the North Sea (focusing mainly on its southern Bay) through :

- The characterization of their genetic structure and diversity: the genetic structure of these populations is investigated using four complementary genetic markers (part of the PhD thesis project of M. Fontaine, in collaboration with Dr J. Michaux; B22 Zoogeography, University of Liege)
- A better understanding of their feeding ecology: the determination of stable carbon and nitrogen signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in the muscles and blood of harbour porpoises and harbour seals provide some further insights on their respective diets (part of the PhD thesis project of O. Drouget, Laboratory for Oceanology).
- The assessment of their susceptibility of being trapped accidentally in fishing nets: the susceptibility to incidental capture will be investigated by both post-mortem investigations and systematic inventory of carcasses in fishing nets.

9.4. Evaluation of the immunotoxicity of mercury, zinc, polychlorobiphenyls and methyl sulfonyl polychlorinated biphenyls on cytokine secretion by marine mammals

Coordination: Krishna Das

The aim of the present research is to evaluate the immunotoxicological risk linked to an exposure of the harbour porpoise and the harbour seal to methyl-mercury (methyl-Hg), zinc (Zn) and to polychloro biphenyls (PCBs). Indeed, in the North Sea, the harbour porpoise (*Phocoena phocoena*) and harbour seal (*Phoca vitulina*) can display high levels of zinc, mercury and polychlorinated biphenyls (PCBs). The project is carried out in close collaboration between Liège University (Dr K. Das), the FTZ (Dr U. Siebert, FTZ, Westkueste, Kiel University, Germany) and the GKSS Forschungszentrum (Dr. S. Fonfara, Germany) in Germany. Harbour seal and harbour

porpoise peripheral mononuclear blood cells (PMBC) are isolated and contaminated with methyl-mercury, zinc, PCBs and MeSO₂-PCBs. The expression of cytokines and the study of the proteome are studied in control and contaminated PMBC.

9.5. Development of a method to identify specific biomarkers related to the effects of dioxins-like compounds on the immune system.

Coordination: Pr. E. Depauw (PhD thesis project of C. Brenez: c.brenez@ulg.ac.be).

Dioxins-type compounds (polychlorodibenzo-para-dioxin PCDDs and polychlorodibenzo-para-furan, PCDFs) accumulated through the food web to top predator such as marine mammals, reaching concentrations that could lead to deleterious effects such as the immunotoxicity. Chemical analysis of contaminants in marine mammal tissues is often used but presents some disadvantages: (1) this approach is limited to compounds having a known toxicity; (2) the cost of analyses is representing a problem and (3) the chemical analysis remains insufficient to predict effects on an individual considering all possible interactions between pollutants and pollutants and also between pollutants and organisms. To complete pollutants analysis, a bio-analytical method could represent a more accurate, cheap and adequate tool by the identification of specific biomarkers. It is for these various reasons that the proteomic became a method of choice to detect proteins expressed differently between the proteins profiles (proteom) before and after exposure to contaminants. Indeed, the various biological processes are directly linked by the proteins, which dynamically are modified and transformed at various levels of intoxication.

The goal of the study is to develop and to validate a bio-analytical method to identify specific biomarkers expressed by lymphocytes after treatment with dioxins-type compounds. The association of cellular cultures with the comparative analysis of the proteome of lymphocytes exposed or not to a mixture of 17 toxic congeners of dioxins and furans (PCDD/Fs) allows to study and to detect cellular functions and proteins modification. The method is developed for human PBMCs and should be applied for marine mammals lymphocytes.

9.6. The influence of active SONARs on marine mammals

Coordination: Mike Carron, NATO Undersea Research Centre

Part of the PhD thesis of Isabelle Noirot (contact: isa_noirot@hotmail.com, PhD advisor: Pr Jean-Marie Bouqueneau)

The following organizations are participating in the project: NATO Undersea Research Centre (La Spezia, Italy), WHOI (USA), CIBRA (Italy), Belgian Defence and MUMM (Belgium).

The aim of the project is to identify possible impacts of active sonars (Low Frequency Active Sonar (LFAS) and mid-FAS) on marine mammals, determine possible options and mitigation procedures for the future, and network with key players in maritime matters. Mitigation procedures should be applied during the use of active SONAR, until thorough knowledge exists about the impacts of man made sounds on marine mammals. These Marine Mammal Risk Mitigation procedures are developed and implemented in the annual SIRENA cruises executed in the Mediterranean Sea (NATO URC). These cruises include carefully controlled acoustic exposure experiments on sperm whales to help understand impacts of LFAS and Mid FAS on this species.

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