North Sea seabirds and marine mammals: pathology and ecotoxicology

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General introduction

Over the past five years several major priority research actions were taken by the federal authorities within the framework of the program 'Sustainable Management of the North Sea'. In particular, the study of the health of the population of seabirds and marine mammals along the Belgian coast, its temporal evolution, the observed pathologies and contaminations as well as the possible causes of unusual mortalities were investigated by a multidisciplinary group of scientists backed up by a solid technical team, the MARIN group (Marine Animals Research and Intervention Network).

The Southern North Sea and in particular the Flemish Banks support a large population of wintering seabirds. During this period, many of them are found stranded dead or moribund on the shore. On the contrary, marine mammals are less numerous in the area and can be found stranded all year round. Oiling, however recently declining (Seys et al., submitted a) was found to be the major direct cause of death for at least half of the beached birds. For marine mammals different lesions and diseases leading to a progressive debilitation of the individuals were observed.

However, indirect causes of the death of individuals of these two top predator groups, most often combined as a succession of stressful conditions (bad weather conditions, scarcity of food, starvation, contamination, diseases, ...) were still debatable and needed further research. This was the main objective of the scientific research of the MARIN group within the framework of the programme 'Sustainable Management of the North Sea'.

Seabirds

Evidence of pollutants accumulation and possible detrimental effects in wild bird species has received great attention during the last decades. A vast majority of these studies referred to robust individuals but not much research work has been devoted to the study of the potential detrimental effects of pollutants on debilitated specimens. Stranded debilitated seabirds represent a choice material offering a wide range of conditions often closely related. They are also likely to be more susceptible to the contaminants' potential detrimental effects compared to laboratory animals as they have to face a range of other (natural and anthropogenic) stressors (Nicholson et al. 1983 ; Foulkes, 1990). This study presents a first attempt to clarify the potential existing links between pathological and toxicological findings and precise the role of heavy metals and organochlorines as additional debilitating agents in a population of wintering common guillemots found stranded at the Belgian coast (Offringa et al. 1995,1996).

Stranded common guillemots collected on our coasts most likely represent only a small fraction of the total population at sea during the winter as not all dying birds at sea are recovered on beaches (Piatt and Ford, 1996; Flint and Fowler, 1998). However, a comparison with the population found wintering in the southern North sea is difficult as an unknown percentage of the individuals at sea will eventually strand on our coasts while the

others either sink, are eaten by scavengers or simply drift away. The individuals collected at the Belgian coast are believed to originate mainly from the north-eastern Scottish colonies.

To evaluate the chance of recovery of the corpses drifting experiments were carried out off the Belgian coast. Results indicated a strong influence of the wind climate (direction and velocity) in the days after the dropping: the closer to the coast the corpses are released, the higher the probability that they will wash ashore. In addition, persistence experiments demonstrated that a bird corpse remains on the beach for 5.8 – 13.3 days, with the minimal value probably being the more realistic one (Seys, submitted b).

Stranded specimens presented evident signs of a progressive debilitation and were recovered in a general very poor health status. At necropsy, among other findings, a large majority of these common guillemots presented clear signs of a general emaciation characterised by a mild to severe atrophy of the pectoral muscles and complete absence of subcutaneous and/or abdominal fat deposits (cachectic status) (Jauniaux et al. 1997, 1998). These features largely confirm that a large majority of the common guillemots had to rely on their protein reserves as an energy source prior to dying. Other frequent lesions considered as most common cause of death was **a**cute **h**emorrhagic **g**astro**e**nteropathy (AHGE).

In this context, we examined the organochlorines and heavy metal levels in the tissues of cachectic versus non cachectic guillemots. The cachectic birds clearly displayed higher renal and hepatic Cu, Zn, total and inorganic Hg and PCBs levels. These differences became even more striking when taking into account the different categories attributed to the cachectic status: the more cachectic the bird the higher the Cu and Zn levels found in the tissues. In addition, when statistically testing the potential influence of different variables (explanatory variables : sex, age, presence or absence of oiling, presence or absence of - ahGe - and cachexia severity) on heavy metal levels (response variables), using a multiple regression analysis, the cachectic status has been shown to significantly affect hepatic, renal and pectoral muscle Cu and Zn concentrations (Debacker et al. 1997, 2000).

In response to combined environmental stressors (e.g. cold weather, starvation, oiling, ...), the common guillemot's body and organs weight were shown to significantly decrease in cachectic individuals. As a result of this general weight loss, trace elements and organochlorine concentrations significantly increase in the tissues. Despite the tissues' weight loss, total lipid contents of both liver and pectoral muscles also decreased significantly underlining the use of fat reserves as energy source prior to protein catabolism. These results suggested that a general redistribution of heavy in the organs is likely to occur as a result of prolonged starvation and protein catabolism.

Compared to guillemots collected in more preserved areas of the North Sea, specimens collected at the Belgian coast were shown to display higher concentrations of Cu, Zn and total Hg. A specific study of Hg and organochlorines (total PCBs and p,p'-DDE) concentrations in the same individuals demonstrated that significantly higher levels of these contaminants were detected in the common guillemots while wintering in the southern North Sea (Joiris et al. 1997 ; Tapia, 1998). The authors discussed both a seasonal cyclic contamination-decontamination pattern and higher anthropogenic contaminant inputs (for Hg and organochlorines) in the southern North Sea. Although no seasonal pattern was observed for either Cu, Zn, Fe and Cd in our sample, evidence of a more contaminated Southern North Sea appeared while comparing heavy metal levels between common

guillemots collected in Brittany (France), following Erika's oil spill to those collected at the Belgian coast.

A set of experiments using the common quail was developed to test the combined effect of contamination (using Cu, Zn and CH_3H_g) and starvation. A cachectic status was shown to develop in half of the quails which were both contaminated and fasted while the fasted non contaminated counterparts did not display any cachectic characteristics. In addition, higher heavy metal levels were detected in the tissues of the cachectic quails (fasted and contaminated) compared to non cachectic specimens of the same group. These results tend to suggest that, although cachexia linked to starvation clearly influenced heavy metal levels in the tissues, those encountered high levels could well, in turn, be active participants favouring a generalised debilitated body condition (Debacker et al. 2001a).

In this general described context, examining the metals' speciation - and more specifically their possible binding to metallothioneins (MTs) - became of interest. The results suggest that MT synthesis could be viewed as a general response to stressing conditions for stranded common guillemots, as it appears to offer a cytoprotective effect against free Cu ions toxicity (Debacker et al. 2001b).

Marine mammals

The Southern North Sea is part of the distribution area of at least four marine mammal species : the harbour porpoise, Phoceona phocoena, the harbour seal *Phoca vitulina*, the grey seal *Halichoerus grypus* and the white-beaked dolphin *Lagenorhynchus albirostris*. The harbour porpoise is by far the most common species in the Northeast Atlantic and the North Sea (Hammond et al. 1995). Other species like white-sided dolphins Leucopterus acutus, hooded seals *Cystophora cristata*, sperm whales *Physeter macrocephalus* and fin whales *Balaenoptera physalus* can be sighted or occasionally found stranded but are still considered as very rare in the North Sea and especially in its Southern Bight (Camphuysen and Winter, 1995 ; Hammond et al. 1995).

Between 1997 and 2001, 77 porpoises were necropsied . The annual distribution was very irregular with major rises in 1999 and 2001. The most common findings were severe emaciation (60 %), acute bronchopneumonia (49 %), and extended multisystemic parasitosis (51 %). It was usual to find more than one apparent cause of death in one porpoise, fatal conditions being closely interwoven. Severe emaciation was characterised by weight loss, reduced blubber layer thickness and dorsal muscle atrophy. Acute bronchopneumonia was characterised by large areas of lung consolidation with haemorrhagic and/or purulent fluid oozing from the lung parenchyma. Most often, it was associated with massive nematodes infestation in airways. The second most frequent observation was severe and extended parasitosis consisting of heavy infestations of multiple organs with associated lesions of bronchopneumonia, chronic ulcerative gastritis and chronic hepatitis. By-catch in fishing nets was considered to be the cause of death of at least 20 % of the porpoises. Three animals were known by-catches, while others were suspected of having been thrown back into the water after capture (Jauniaux et al. 2002).

Thirty-six harbour seals were necropsied during the 1997-2001 period. The main findings were emaciation with reduction or absence of fat and muscles atrophy, acute bronchopneumonia and enteritis. In addition, 3 seals had evidence of trauma (numerous muscle hematomas and bone fractures). One of these seals was crushed by a motorbike when it was laying on the beach while the origin of the trauma for the 2 others was not determined. At least 3 common seals had drowned in fishing gear. Seven seals were infected by a morbillivirus during the 1998 summer (Jauniaux et al. 2001). In addition, five other cases were diagnosed positive after the 1998 epizootia by immunohistochemistry (n = 2) or RT-PCR (n = 5).

Between 1997 and 2001, 4 fin whales were necropsied, one being stranded on the Belgian coast (Raversijde, 1997), one being trapped in a Dutch harbour (Vlissingen, 2001) and two coming ashore in northern part of France (Wimereux, 1998; Dunkerque, 1999). All were immature females, aged about 1 year. Two whales had evidence of morbillivirus infections obtained by immunohistochemistry and confirmed by RT-PCR, ultrastructural investigations, and serology.

Marine mammals appear to be potentially valuable indicators of the level of persistent contaminants accumulated in the marine environment. According to their top position in the trophic network, their long life span and their long biological half-time of elimination of pollutants, these animals can accumulate high levels of these compounds. Several investigations have been carried out in an attempt to evaluate organic contaminant effects at ambient environmental levels (Reijnders et al. 1986). However, few studies have tried to link marine mammal health status and metal level within the North Sea and adjacent areas (Hÿvarinen and Sipilä, 1984; Siebert et al. 1999; Bennet et al. 2001).

PCB concentrations in blubber of harbour porpoises from the Southern North Sea, expressed as ICES7 ranged between 1.25 and 36 µg/g on a fresh weight basis (n = 18), with CBs 153, 138 and 180 covering over 90% of ICES7 in all tissues and CB 153 in particular ranging between 33 and 53 %. These values are of a same order as the ones recorded earlier for the same area (Van Scheppingen et al. 1999) in harbour porpoises stranded on the English coasts (Kuiken et al. 1993; Jepson et al. 1999) as well as in *e.g.* seals from the polluted Bay of St. Laurent, Canada (Bernt et al. 1999). A relation with age is not distinct, though all the older females show low concentrations as a result of the excretion of organochlorine through lactation.

Heavy metal concentrations depend not only on the environment contamination but also on several other biological factors such as the diet or the age (Das et al. 2002 a). Marine mammals display strong interspecific Cd concentrations. The highest Cd concentrations were observed in the kidney of the oceanic feeders such as sperm whales (Holsbeek et al. 1999; Das et al. 2000c). Cd values were also very high for the 2 white-sided dolphins and the hooded seal but were relatively low in white-beaked dolphin, harbour porpoise, grey and harbour seal kidneys. The high concentrations in the hooded seal, sperm whales and white-sided dolphins are likely to be diet related as teuthophageous marine mammals display elevated Cd concentrations in their livers and kidneys (Bustamante et al. 1998).

Trace metal concentrations measured in harbour porpoises stranded on the Southern North Sea coasts were higher compared with data available from the literature. It appears that harbour porpoises from the North Sea can display high hepatic Zn and Cu

concentrations when compared to porpoises from the Baltic or the Black Sea. Siebert et al.(1999) observed that harbour porpoises from North Sea are carrying a significant burden of mercury. The higher mercury content in organs from harbour porpoises from the North Sea indicated that mercury is a more important threat for animals of this region than for animals of the Baltic Sea.

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

Potential effects of toxic metals cannot be tested in free-living cetaceans because experimental manipulations are undesirable. One approach to this problem is to carry out systematic post-mortem investigations to establish the disease status of contaminated animals in a relatively large number of individuals from the same species.

Trace metal concentrations were compared between emaciated and non-emaciated porpoises. Hepatic Zn and Se concentrations measured were significantly higher in emaciated juveniles compared to non-emaciated ones. In adults, trace metal levels were similar between emaciated and non-emaciated porpoises. Other trace metal concentrations (Cd, Cu, Ni, Cr, Pb, Hg, Fe) were similar between emaciated and non-emaciated porpoises both for adults and juveniles ($p > 0.1$).

High Zn concentrations encountered in emaciated juvenile porpoises could be related to a redistribution of zinc from other organs such as liver or muscle (Bennet et al. 2001). Indeed, food deprivation in rat can lead to a redistribution of the hepatic Zn due to protein catabolism and the increase of hepatic zinc concentration is related to a loss of the liver mass (Krämer, 1993). However during the emaciation process displayed by porpoises, no loss of liver mass has been observed (Siebert, *pers. comm.*), indicating that the increase of hepatic Zn concentration might be related to muscle rather than liver protein catabolism. Although emaciation can influence the heavy-metal levels, the high levels encountered in the porpoises from the Southern North Sea could possibly favour a debilitating process leading to emaciation.

Metallothioneins have been shown to play a key role in marine mammal metal homeostasis (Das et al. 2000a, 2002 b). Further investigations on MT function have been carried out on harbour porpoises displaying a good conservation state. Total proteins have been measured in the livers and it appears that MTs represent 1.3% of the total protein concentrations. Despite this low percentage, MTs appear to play a key role as binding 50 % of the total hepatic Zn. Moreover, when Zn increases in the liver, its percentage bound to MTs increases suggesting that these proteins might take in charge the Zn overload resulting from the emaciation process. MTs are also involved in renal Cd detoxication as they bind 56% of the total renal Cd. Both in livers and kidney, MTs appear to have a weak role in Hg detoxication as this metal is distributed mainly in the pellet.

General conclusions

Whithin the North Sea, both marine mammals and seabirds have to face a wide variety of natural and human induced stressful conditions. Apart from oiling (acute and chronic) which remains an important source of mortality for seabirds, starvation has been shown to greatly influence the individuals general health status as well as its heavy metals levels. For porpoises, fatal conditions observed in this study were generally similar to those described from other regions of the North Sea. Entanglement in fishing nets was an important cause of death in our series although lower compared to other countries. In our analysis, it seems that emaciation and severe parasitosis predispose porpoise to progressive debilitation, leading to pneumonia. Acute bronchopneumonia was considered to be the cause of death of 49 % of the porpoises. Infectious diseases were diagnosed on half of the seals, most of them being infected by morbillivirus. It seems to be the first evidence of distemper in the North Sea since the 1988 epizootia. The other half showed evidences by-catch or severe trauma. Similarly, morbillivirus infection was also observed in 2 out of 4 fin whales. To the authors' knowledge, this is the first firm report of morbillivirus disease in baleen whales.

While starving and re-adjusting its whole metabolism and certainly increasing it to face its demanding energy requirements, the common guillemot also undergoes a general redistribution of its Cu, Zn and organochlorine contents. This results in increasing circulating contaminants which are then re-routed towards target organs where they reach levels which, in turn, could well favour a generalised lessened body condition.

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

In view of these different points, re-assessing the role of pollutants in both debilitated stranded common guillemots and marine mammals gave a first insight of the complex interactions existing between contaminants, their potential detrimental effects and the individuals' general fitness. Although not at risk on a toxicological basis under normal conditions (robustness) these two top predators could well be adversely affected by their contaminant levels with degrading body condition.

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