CHAPTER 2 - LITERATURE REVIEW

2.1 Birds as bioindicators – an introduction

Heavy metals, both essential and non-essential, have the potential to be toxic to organisms above a certain threshold concentration. The threshold concentration depends on various factors, including the bird species, the metal species, bioavailability and exposure (Burger, 1993). It is thus important to keep several criteria in mind when selecting the bird species that will be used in the biomonitoring program. Most of these criteria are based on practical aspects, such as the collecting of the samples. In general, bioindicators (including birds) should be very common and abundant and have a wide distribution. They must be easy to catch on a routine basis. Using species from the different trophic levels should be done if possible, as this shows what is going on in the whole ecosystem. Species that represent all the different exposure routes in the ecosystem should also be chosen. To determine the contaminant levels of a specific site, non-migratory species should be chosen. The organisms must also be easily identifiable (Burger, 1993).

According to Dmowski (1999) the criteria for an ideal bioindicator limit the number of bird species that could be used. The requirements include:

- Widespread occurrence.
- Clearly identified individual territory size and territory fidelity.
- Common occurrence and easy capturing.
- Homogeneity of the material and standardisation possibility.
- Individual size large enough, if monitoring is to be based on the tissues of single animals.
- Well known biology of the species.
- Breeding possibility for laboratory tests.
- Bioaccumulation of xenobiotics or other clear reaction to certain xenobiotics.

Most studies just focused on the level of contaminants in various organs, eggs and/or feathers of the birds (Sawicka-Kapusta *et al.,* 1986). Although crucial in the overall impact study, effect studies are scarce. There are only a few examples in literature (e.g. the effects of PCBs on egg shell thickness in birds of prey) that have clearly linked contaminant burdens in birds to specific biological or ecological effects. Mora (2002) studied the metal content in the eggs of various passerines to determine if they had an effect on the hatching success. The few examples from southern Africa include the heavy metal levels in feathers from seabirds in Namibia (Burger and Gochfeld, 2000a) and organochlorines in the blood and tissues of vultures (Van Wyk *et al.,* 2001). It is clear that for these important species cause-effect relationships are essential for a better quantification of ecological impact.

2.2 Metal biomonitoring using feathers

During the recent years the use of feathers in biomonitoring programmes has increased. The European Union already uses feathers as an integrated part of their marine biomonitoring programmes using EcoQOs (ICES, 2003).

Burger (1993) gave several reasons why feathers are useful for determining metal levels in birds:

- Metals are deposited in the feathers during their formation.
- Metals are only deposited during the short period of feather growth and are thus a record of blood levels at the time of feather formation.
- Feathers are easy to collect, as they can be plucked from both live and dead specimens.
- Feathers are collected from live birds without causing undue damage.
- Collection from live birds is a non-invasive procedure that can be performed by field assistants.
- Feathers can easily be stored in metal-free containers and do not require refrigeration.
- Metal profiles are not easily disrupted by long-term storage.

There are also some disadvantages to using feathers. They mainly concern the assumptions made about the metal deposition in the feathers. Points to keep in mind are:

- The stability of Metal Profiles in the feathers.
- External contamination.
- Metal level variations among feathers and feather parts.
- Molt and timing of sampling.

These problems but can however be overcome by good project planning (Burger, 1993).

In the past feathers of mostly seabirds and bird of prey have been used as bioindicators of metal pollution. Dauwe *et al.* (2003) compared the levels of various metals in the primary wing feathers of several birds of prey. Pain *et al.* (2005) discussed the potential significance of lead poisoning in Spanish Imperial Eagles (*Aquila adalberti*) and other raptors and also recommended measures for its reduction. The concentrations of various metals in the feathers of Laggar Falcons (*Falco jugger*) in Pakistan did not differ significantly between birds of different gender or age (Movalli, 2000). The metal levels in feathers of various tern species (Family Sternidae) from islands in the North Pacific were studied by Burger *et al.* (2001). Burger and Gochfeld (2000b) indicated that Black-footed Albatrosses (*Diomedea nigripes*) had higher levels of heavy metals in their feathers than Laysan Albatrosses (*Diomedea immutabilis*).

Studies on waterfowl (family Anatidae) and small waders have also been done, mainly focusing on lead. Little attention has been given to passerines or gallinaceous birds. Recent studies done in Europe have been trying to determine the use of passerines as biomonitoring species. The use of Black-billed Magpie (*Pica pica*) tail feathers in Poland as bioindicators were investigated (Dmowski, 1999; Dmowski and Golimowski, 1993). In Belgium a number of studies have been done on the use of tits (family Paridae) as bioindicators, for both metal and organic pollution. Two species were chosen, the Blue Tit (*Parus caerulescens*) and the Great Tit (*Parus major*). Studies included the level of metals in feathers along a pollution gradient (Dauwe *et al.,* 2002a; Eens *et al.,* 1999; Janssens *et al.,* 2001), and the relationship between metal concentrations in nestlings and their environment and their food (Dauwe *et al.,* 2004). Most studies were also conducted in the northern hemisphere. Studies in the southern hemisphere were done in Namibia (Burger and Gochfeld, 2000a) and Chile (Ochoa-acuña *et al.,* 2002).

Very little laboratory studies have been done on the effects of metals on birds. An example is the exposure of Zebra Finch (*Taeniopygia guttatta*) to lead to determine if there is a dose-response relationship in the metal levels of the feathers (Dauwe *et al.,* 2002b).

2.3 Metal biomonitoring using biomarkers (non-haematological)

Biomarkers are an integral part of biomonitoring. Biomarkers in birds have been used in the past as part of biomonitoring programmes (Cordi *et al.,* 1997). These have included several enzymes, DNA studies and behavioural aspects of the organisms. There are not many studies done on the use of biomarkers for metal contamination using birds. Most studies focus on fish (Van der Oost *et al.,* 2003) or invertebrates (Smolders *et al.,* 2005).

Since any changes in DNA may have lasting and profound consequences on organisms, DNA damage is a good biomarker to test the genotoxicity of pollutants (Connell *et al.,* 1999). Understanding the impact of a toxicant on the genetic level of an organism is important as this may be the earliest warning to a potential impact on the population or communities of organisms in the ecosystem. Although DNA damage is a non-specific assay, it is applicable to many cell types and has a high sensitivity (Mitchelmore and Chipman, 1998). DNA damage is as a good biomarker for the assessment of the genotoxic properties of pollutants and has been successfully used in molluscs (Lee and Steinert, 2003; Moolman, 2004), fish (Hoff *et al.,* 2003; Lee and Steinert, 2003) and birds (Pastor *et al.,* 2001).

Various anti-oxidant biomarkers are used in biomonitoring studies. Catalase is an anti-oxidant enzyme that reduces H_2O_2 into H_2O and O_2. When the organism is under oxidative stress, the levels of catalase activity will rise, until a point when the system cannot cope and the activity drops again. Many studies on the level of catalase activity have been done on poultry, mainly to see what the effect of various compounds is on chickens (Yuming *et al.,* 2003; Wu and Squires, 1997). Catalase activity has also been successfully used as biomarker in pollution monitoring in molluscs (Gravato *et al.,* 2005; Moolman, 2004), fish (Almeida *et al.,* 2005; Ferreira *et al.,* 2005) and mice (Bonilla-Valverde *et al.,* 2004).

The reduced glutathione content (GSH) has been used as a biomarker of oxidative stress in humans and laboratory animals, but rarely in wild birds (Isaksson *et al.,* 2005). Functions of glutathione include maintenance of cell membrane integrity, drug and chemical metabolism and protection from oxidative stress (Rogers and Hunter, 2001). Examples of studies on other animals include molluscs (Gravato *et al.,* 2005; Moolman, 2004) and fish (Sayeed *et al.,* 2003). It has been used as part of a quality control measure in broiler chicken meat (Jahan *et al.,* 2004). It has not been used on birds a lot in pollution monitoring. One example is the use of GSH in the monitoring of stress Great Tits in Sweden (Isaksson *et al.,* 2005). Hoffman (2002) used GSH levels to determine the role of selenium in the oxidative stress in aquatic birds.

2.4 Haematology as health indicators in avian studies

Avian and mammalian haematology is similar in many ways. They differ though in some ways (Campbell and Dein, 1984). The most important are that the erythrocytes of birds have a nucleus when mature. Instead of platelets, birds have nucleated thrombocytes. The initial pathways for blood coagulation in birds and mammals differ considerably. Birds also have heterophils instead of neutrophils. Mammalian blood has higher levels of plasma albumin than avian blood. Avian bone marrow contains a large amount of lymphatic tissue as compared to that of mammals. Avian haemoglobin contains inositolpentophosphate instead of 2,3-diphosphoglycerate as in mammals. Erythrocyte maturation is also much faster in birds.

Studies on avian haematology started out mostly as studies on chickens, ducks and other domestic birds. Hemm and Carlton (1967) reviewed the haematology of various duck species, determining the normal values. These early studies mostly focussed on developing haematological techniques for birds and getting normal values. Later they included studies relating to the effect of disease or certain chemicals affecting the overall health of the birds. Ots *et al.* (1998) devised a health index based on haematological parameters for Great Tits. Ferrer *et al.* (1987) showed that there is a difference between the blood values of individual captive birds of prey in good and bad states of nutrition.

Studies later focussed on combining the determination normal values for avian haematology with the responses to different substances and infections. The effect of testosterone on House sparrows (*Passer domesticus*) was shown by Puerta *et al.* (1995). This study also contributed valuable normal haematological values for small passerines. Booth and Elliott (2003) also studied the haematological responses of passerines to haematozoa. The normal values for various species of Neotropical passerines were also determined in the study.

Since it is easier to get blood from larger birds, studies tended to focus on larger non-passerine birds. The studies were mainly carried out on captive birds in zoos as this was practically easier. The first systematic comparative study of the normal haematological values for game birds was done by Balasch *et al.* (1973). They compared six species of Galliformes from the Barcelona Zoo. Puerta *et al.* (1991) studied the haematology of six captive Great White Pelicans (*Pelecanus onocrotalus*) and determined normal values for the different haematological parameters.

Studies to determine if there is seasonal, sexual and age-related variation in the blood chemistry of captive Brown Pelicans (*Pelecanus occidentalis*), also gave information about some haematological parameters (Wolf *et al.,* 1985). It showed a variation in the haematocrit due to age or sex. The haematology of captive Chilean Flamingos (*Phoenicopterus chilensis*) was studied by Puerta *et al.* (1989), in which they also showed there is not much difference between the haematology of males and females. Polo *et al.* (1994), on the other hand, observed some significantly differences between age groups and some similarities between related birds while studying the haematology of captive waders.

Studies on wild large flying and aquatic birds (Balasch *et al.,* 1974) were done to determine whether haematological values would differ according to the requirements for flight or diving. No clear differences were found. Another study on Great White Pelicans used blood chemistry and haematocrit values to determine if migratory birds need to stop over in Israel. They used both captive and migrating birds for the study (Shmueli *et al.,* 2000).

Various other studies used wild, free-living birds. Normal haematological values from wild African White-backed Vulture (*Pseudogyps africanus*) nestlings were established by Van Wyk *et al.* (1998). Lavin *et al.* (1992) found that there was no difference between the haematology of males and females of wild Marsh Harrier (*Circus aeruginosus*).

Studies on wild young Great Bustards (*Otis tarda*) (Alonso *et al.,* 1990) showed that there was a difference in the haematology of the young and adult birds. The same was done on wild White Storks (*Ciconia ciconia*) (Alonso *et al.,* 1991), which also showed that there was a difference between values of adults and young. Significant differences were also found for the blood chemistry between adult and juvenile wild White Spoonbills (*Platalea leucorodia*) (de le Court *et al.,* 1995). Melrose and Nicol (1992) compared values they obtained for the haematology, red cell metabolism and blood chemistry of wild, fledgling Black-faced Cormorants (*Leucocarbo fuscescens*) with other seabirds. Aguilera *et al.* (1993) studied the blood chemistry of three penguin species and found no apparent variation in penguin normal blood chemistry values that could be interpreted as an adaptation to the special environmental conditions of the Antarctic. Puerta *et al.* (1992) did a comparative study between the haematology of captive and wild Greater Flamingos (*Phoenicopterus ruber*). Later they focussed on the physiological requirements or adaptations of birds.

In veterinary sciences several haematological parameters are used for the determination of the physical condition of birds. Campbell and Dein (1984) discussed these in their practical guide to avian haematology. According to them haematocrit is the quickest and most practical method for evaluating the erythrocyte status of a bird. The haematocrit indicates the level of anaemia or dehydration, which are both difficult to evaluate by physical examination alone. Most birds have a haematocrit value ranging from 35 to 55 %, where a value below the range suggests anaemia and one above dehydration. Anaemia may be caused due to haemorrhage, destruction of erythrocytes or decreased production of erythrocytes. The haematocrit is affected by both the number and size of the erythrocytes. Birds and mammals respond to hypoxia by increasing the number of erythrocytes, with acute stages reaching haematocrit values of 60 to 80% in birds (Campbell and Dein, 1984). The haematocrit is also influenced by changes in the plasma volume that does not affect the number of cells, e.g. and increase (haemodilution) or decrease (haemoconcentration) in the plasma volume. Hypothermia is one of the causes of haemoconcentration.

The number of erythrocytes is influenced in general by season, time of the day, environmental temperature, laying cycle, age, sex, hormonal influences and hypoxia (Campbell and Dein, 1984). Examples of hormonal influences include a decrease of erythrocytes due to estrogen and an increase of numbers due to androgens and thyroxin. Avian haemoglobin has four iron-containing haeme units like mammalian haemoglobin, but the globulins differ.

Even though haematological parameters are good indicators of the overall condition of the bird (Campbell and Dein, 1984), very little studies focused on the use of haematological parameters of birds for biomonitoring of environmental conditions. Studies in India concerning pesticides are some of the few examples of haematological studies used in biomonitoring programmes. The haematological changes produced by Lindane (γ-HCH) in six species of birds were investigated as to indicate whether it can be used as an early warning system for pesticide toxicity (Mandal *et al.,* 1986). The study also reported very important normal values for haematological parameters for passerines, including a weaver species. Lindane induced anaemia, as judged by the reduced erythrocyte count, haematocrit and haemoglobin content. The study also showed that bird species react to chemicals in different levels of intensity. It showed that the early haematological changes induced by Lindane may serve as an early warning for toxicity.

Other examples include Henny and Meeker (1981), who investigated the use of blood plasma levels of DDE as an indication of the effect of DDE on birds of prey. The haematological parameters of two grebe species were investigated as potential biomarkers of mercury in North America (Elbert and Anderson, 1998). The values obtained were correlated to the levels of mercury in blood and various other organs.

Sparling *et al.* (1998) determined the changes in the blood of Mallards (*Anas platyrhynchos*) as bioindicator to exposure to white phosphorus. They proposed haemoglobin as a biomarker of exposure. Pain (1989) did a similar study, trying to find which haematological parameter can be used as predictors of blood lead and indicators of lead poisoning in the American Black Duck (*Anas rubripes*).

2.5 Ecological Quality Objectives

In general Environmental Quality Criteria (EQCs) for most countries are based on toxicity data from invertebrate or fish species, which are generated through laboratory tests. Data obtained from birds are often not used or available. These EQCs are generally derived by extrapolation. At present, such extrapolation is mainly based on safety or uncertainty factors that are applied to (mainly laboratory-based) toxicity data. In order to prevent under protective quality standards, application factors ranging from 10 to 1000 are applied. However, nowadays the final quality standards are derived without accounting for important co-variables or species that could seriously affect the final outcome of risk assessment procedures (Pascoe *et al.,* 2000).

For birds, laws and guidelines regarding the protection of birds are mainly focussed on hunting and habitat protection (Gillies, 1998). Although these two are important, the adverse impact of pollutants on birds should not be ignored. A good illustration is the bioaccumulation of heavy metals in birds, e.g. lead in waterfowl (Mute Swans in the United Kingdom – Perrins *et al.,* 2003). In these cases the heavy metal levels did not affect organisms at lower trophic levels, but had a very negative effect on both the reproduction and survival of these birds.

In the past birds have not been used in biomonitoring programmes. More and more, however, birds are suggested to become an integral part of biomonitoring campaigns. The International Council for the Exploration of the Sea (ICES) discussed the importance of the use of seabirds in the monitoring of marine pollution at the 1999 meeting of the Working Group on Seabird Ecology (ICES, 1999).

The ICES recently proposed various EcoQOs, which are being used in environmental monitoring (ICES, 1999). Seabirds have been identified as EcoQOs for use in the North Sea (ICES, 2001). The monitoring of contaminants forms a category of EcoQOs within the EcoQOs framework designed by the ICES. However, due to a lack of available data, reference and target values, however, still need to be set. For a limited number of chemicals (mercury and organochlorines) EcoQOs have been proposed (ICES, 2003; ICES, 2004). For certain parts of the North Sea some data have already been gathered and the EcoQOs proposed.

EcoQOs have several key features (ICES, 1999), listed below:

- They should improve or maintain ecological quality, should be sensitive to a manageable human activity and relatively easy to measure.
- They must also show a high response to specific human activity and be relatively tightly linked in time to the activity.
- The EcoQOs must be measurable in large proportion of the area where the EcoQO is applied.
- They must also be able to be measured over a number of years to provide baseline information and a setting of realistic objectives.
- The EcoQOs may relate only to species for which the objective is being set or be an indicator of a wider ecological condition.

With the above mentioned in mind the ICES devised several categories for the EcoQOs for seabirds. According to the Working Group on Seabird Ecology's 1999 report (ICES, 1999), the categories are as follows:

a) Contaminants
    i. Oil
    ii. Other contaminants
b) Eutrophication
c) Litter
    i. Plastic particles

d) Fisheries
    i. By-catch of seabirds
    ii. Harvesting of seabird food
    iii. Provision of seabird food
e) Mariculture
f) Habitats and ecosystem health

For seabirds, two species were chosen was key species, the Common Tern (*Sterna hirundo*) and the Palearctic Oystercatcher (*Haematopoda ostralegus*). In the category of Contaminants, the contaminants mentioned are oil, mercury and organochlorines.

To determine the reference levels, museums specimens were used. Usually birds that were collected before 1900 were used (ICES, 2003). This can be done since the levels of metals in feathers are usually stable (Burger, 1993). The target levels are then set as a value not higher than 1.5 times the reference values. In the setting of target levels, there are no hard scientific facts or rules; they are just a matter of societal choice and public acceptability (ICES, 1999).

The setting of EcoQOs is still in the beginning phase and should be developed further for other regions and also for other habitat types and for other species. Key species should be chosen for each ecosystem and more categories for other contaminants, especially metals, should be investigated.