Seabirds as Monitors of Mercury Contamination in the Portuguese Atlantic

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In memory of Professor José Ávila Martins, for his contribute to strengthen marine research in the Azores. This work exists because of his wisdom on prominence of environmental issues.

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CONTENTS*

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SUMMARY

1. The past and present status, ecology and conservation of the Azores seabirds were analysed. Historical population trends inferred from 16th and 17th century chronicles indicate dramatic declines for most species. Current community structure and functioning were reviewed and updated to provide a framework for their use as monitors of mercury in the marine environment. Ecological segregation among Procellariiformes involves partitioning between epipelagic and mesopelagic food resources.

2. A review of mercury in seabirds was undertaken. Mercury dynamics and factors causing inter- and intra-specific variations in mercury concentrations are discussed. Methodological considerations relating to the use of seabird tissues as monitors for mercury are discussed and recent studies on geographical and temporal changes are summarised.

3. The breeding biology of Madeiran storm petrel *Oceanodroma castro* in the Azores was studied. Two dimorphic and temporally segregated populations (hot and cool seasons) breed annually and sympatrically on Graciosa island. The hot season birds are 10% smaller in egg and body mass but are longer-winged and longer-tailed than cool season birds. The two groups were readily separated by discriminant analysis, with over 85% of the individuals being correctly assigned to the population they belong. Possible ecological constraints and adaptations promoting divergence are discussed. These populations may represent a case of sympatric speciation with time as the isolating mechanism.

4. The moult phenology of adult Cory's Shearwater *Calonectris diomedea borealis* overlaps a great extent with the breeding season. Primary renewal starts five weeks after peak hatching and seven weeks before peak fledging. Such an overlap of wing moult and breeding has never before been described in a pelagic and highly migratory seabird such as the Cory's Shearwater.

5. Intra-specific variations of mercury concentrations in tissues of seabirds from the Portuguese Atlantic were analysed, providing a baseline 'noise' for their use as monitors. Examination of relationships between mercury concentrations in blood and plumage of adults and chicks provided validation of current models for mercury dynamics in birds and elucidation of most intra-specific patterns of variation in mercury concentrations.

6. Moult emerged as the main explanation for variations of mercury concentrations in contour feathers and blood of adults. In chicks, mercury concentrations in plumage and blood decline dramatically with age. This growth dilution effect results from highly transient blood concentrations due to an imbalance between mercury dietary intake and co-accumulation in developing plumage and growing internal tissues.

7. Linear relationships between mercury concentrations in blood and growing feathers were found in chicks and adults. The slopes of such relationships were consistent over a wide range of concentrations and indicate a standard feather:blood partition coefficient of about 3.

8. Mercury concentrations in short-lived fish of low trophic levels were investigated in relation to their vertical distribution. Concentrations were positively correlated with median daytime depth, increasing by four-fold from epipelagic (<100m) to mesopelagic species (>300m). This confirmed enhanced bioaccumulation of mercury in marine mesopelagic environments due to methylmercury production in sub-thermocline low oxygen seawater.

9. The relationship between mercury in diet and seabird body burdens of mercury was investigated. Seabirds specialised on mesopelagic prey show mercury burdens about four-fold higher than those feeding predominantly on epipelagic prey. Ecological segregation in seabird communities resulted in many parts of the world into feeding specialisation on pelagic or mesopelagic organisms, and thus seabirds may be used for monitoring mercury contamination within and between vertical compartments of marine ecosystems.

10. Methylmercury trophic transfer between fish/squid and seabird feathers leads to biomagnification factors around 150x, the highest ever reported for biomonitors between consecutive trophic levels and strengthens the potential of seabird feathers as mercury monitoring units.

11. Experimental evaluation of methylmercury kinetics, dose-responses, excretion and toxicity in free-living adult Cory's shearwater was undertaken using a non-destructive multi-tissue (blood, feathers and eggs) approach. Elimination in the blood comprises an initial fast phase, with half-time of ca. 1 day, and a slow terminal phase. The half-time in the latter phase varied between ca. 44 days and

65 days, for adults exposed, respectively, a month or two months and an half before start of moult, which confirms moult as a crucial factor in methylmercury elimination. Half-times were independent of dose over an eight-fold range (0.3-2.5 μg/g). The average fraction of ingested methylmercury deposited in the blood volume was 10.4%.

12. A relationship between steady-state blood concentrations and dietary intake of methylmercury was derived. Relationships between mercury concentrations in parental blood and levels in eggs and hatchlings were linear and generally independent of dose, providing blood:tissue (eggs, feathers) partition coefficients identical to those observed in controls. Dose-response relationships were linear over the wide range of exposures employed. The kinetic parameters obtained may be used in advanced modelling of the kinetics of methylmercury in adult birds.

13. A sex-related difference in the blood dose-response was observed. Females were subjected to mercury loads 16-20% higher than males but exhibit a lower dose-response by about 10%. The difference is not fully accounted by the 14% excretion of the dose into the egg, and potential, but unidentified, sex-related differences in physiology may be the ultimate cause.

14. The relative contribution of distant and immediate dietary intake of mercury in the excretion into the plumage during the moult cycle was assessed. Excretion rates showed lack of dose-dependency, and were higher (ca. 27% of the intake) in birds exposed during the moult cycle compared to ca. 7% excreted in birds dosed up to two months before the start of the moult cycle. Mercury excretion through the skin in exfoliated epidermal cells that adsorb into plumage was estimated to represent up to a minimum of 6% of the intake.

15. Methylmercury kinetics, dose-responses, excretion and toxicity were experimentally evaluated and compared between small and large free-living Cory's shearwater chicks. Blood methylmercury half-time for the terminal elimination phase was ca. 5.7 days and the average fraction of ingested methylmercury deposited in the blood volume was 12%. The half-time for chicks is much lower than that of adults, stressing the importance of the rapid growth of body tissues and plumage in governing mercury dynamics in chicks. The former kinetic parameters were independent of the age at exposure and were employed to derive

a relationship between steady-state blood concentrations and dietary intake of methylmercury in avian chicks.

16. Blood:plumage relationships were linear, indicating a lack of dose-dependence for blood:plumage partition coefficients. Dose-response relationships were also linear over the wide range of exposures employed. Dose-responses in blood were remarkably similar among small/large chicks and adult Cory's shearwater. That suggests a general dose-response kinetics for methylmercury in avian blood, governed by the volume of the body pool, assuming that the fraction deposited in blood after complete absorption would not differ among species. Excretion rates into the final plumage varied between 42% and 60% of the intake and reflect to a certain extent (possibly up to 5-10%) exogenous contamination with exfoliated cells.

17. Potential sub-lethal toxicity of the doses administered was assessed. No significant responses were found in any of the avian indicators of methylmercury poisoning employed: egg production and hatchability, body condition of adults and growth of chicks. Thus, the current exposure levels provide maximum avian non-observed-adverse-effect-levels (NOAELs) for external symptoms in wild birds.

18. Mercury concentrations in tissues of breeding seabirds were used to infer contemporary spatial patterns in mercury contamination in the epipelagic and mesopelagic environments of the Portuguese Atlantic. Results indicate a many-fold difference in mercury contamination between vertical compartments coupled with an even distribution of contamination in both compartments across most of the study region. That confirms predicted global pollution by mercury due to atmospheric deposition at long distance from emission sources.

19. Mercury concentrations in feather time-series (1886-1994) from seabirds breeding in the sub-tropical North-east Atlantic were used to infer historical trends in mercury contamination in epipelagic and mesopelagic environments. Long-term rates of increase near the apex of the mesopelagic food web (2.9-4.8%.yr^{-1}) correspond to a three-fold amplification of the anthropogenic-derived pulse of mercury comparatively to analogous rates for the epipelagic food web (0.7-1.9%.yr^{-1}).

20. The increases observed in seabirds feeding near the apex of food chains provide an empirical linkage between increasing accumulation of methylmercury in aquatic organisms and anthropogenic influence in the global mercury cycle. The historical trends for the epipelagic environment are consistent with current estimates for global increase in the atmosphere of 1.2-1.5%.yr^{-1}. This coupled with the mesopelagic magnification of historical increases are of concern because of the current public-health problem resulting from widespread incidence of elevated levels of methylmercury in fish.

CHAPTER 1

GENERAL INTRODUCTION

CONTENTS

1.1. MERCURY AS A POLLUTANT

Mercury has been well known as a pollutant for several decades. In the 1960s and 1970s, episodes of methylmercury intoxication in human and wildlife populations have occurred in association with important local discharges of mercury into air or water from point sources and the use of mercurial fungicides (e.g. Borg *et al.* 1969, WHO 1976, Takizawa 1979, WHO 1990). During the 1980s, a new pattern has emerged with regard to mercury pollution, when fish from oligotrophic remote lakes in North America and the Nordic countries were found to contain high concentrations of mercury (Lindqvist *et al.* 1991). That could not be linked to individual emissions of the metal and it has become evident that mercury emissions to air are dispersed on a regional scale, with much of the emitted mercury being deposited within 1,000-2,000 km from the sources (Lindqvist 1994). From the early 1990s to today, concern about human impact on the mercury cycle has increased. Recent studies indicate that the global background of mercury has increased many-fold since industrialisation due to anthropogenic sources (Mason *et al.* 1994a, Hudson *et al.* 1995).

From an environmental health perspective, the main concern regarding mercury pollution is the high consumption of aquatic organisms (particularly fish) with elevated methylmercury (its most toxic form) by humans and wildlife (WHO 1990). Methylmercury intoxication in humans is characterised by effects on the central nervous system and the areas mainly affected are those associated with the sensory, visual, and auditory functions and those concerned with co-ordination (WHO 1976). The developing nervous system of the foetus is more sensitive to methylmercury than the adult, and pre-natal exposure can result in neurotoxic effects in the infant in the absence of effects in the mother (WHO 1990). The exposure thresholds for initial effects in adults are in the ranges of 0.2-0.5 µg/g fresh weight in blood and 50-125 µg/g fw in hair. However, 10-20 µg/g fw in maternal hair during pregnancy is associated with a 5% risk of methylmercury poisoning symptoms in the infant (WHO 1990). Given the current levels of mercury in fish stocks in many areas (e.g. Mediterranean, UNEP/FAO/WHO 1987; Nordic lakes, Lindqvist *et al.* 1991), the thresholds for developing foetuses might be approached or even exceeded in populations that subsist on fish. As a result, public health authorities started to issue blanket health advisories, like the one covering all Michigan's inland lakes in 1989; pregnant women, nursing women, women who intend to have children, and all children aged 15 or under were

advised not to eat large amounts of several fish species that were particularly heavily contaminated with mercury(MDPH 1989).

1.2. ENVIRONMENTAL CYCLING OF MERCURY

For a number of reasons, mercury and its biogeochemical cycle are unique among metals of concern for their potential harmful environmental effects. The metal is chemically distinctive (evaporates to the atmosphere due to its high vapour pressure) and because of its tendency to form strong covalent bonds its behaviour in biological systems is also distinctive (Carty & Malone 1979). Unlike most other metals, mercury is efficiently biotransformed into its most toxic form (methylmercury) in sediments and anoxic waters of lakes and oceans (Beijir & Jernelöv 1979, Topping & Davies 1981). It is the only metal which is consistently biomagnified through the food chain (Windom & Kendall 1979, Kidd *et al.* 1995), i.e. mercury becomes more concentrated through each step in the food chain. As a result of these biogeochemical properties of mercury, elevated exposures tend to be quite natural phenomena (WHO 1990). This suggests that relatively minor perturbations of key portions of the cycle (e.g. atmospheric deposition, accumulation by fish and other predators) could result in major changes in the exposure of, or uptake by, sensitive human or wildlife populations even in remote areas (Lindberg 1987). In this respect, the recent discovery of increased concentrations of methylmercury in oceanic waters below the thermocline (Mason & Fitzgerald 1990, Cossa *et al.* 1994) is noteworthy, given the potential for enhanced accumulation of mercury through the food chain into demersal fishery resources.

The overall global cycle of mercury is fairly clear although a detailed understanding of may mechanisms is lacking (Fitzgerald 1989, Lindqvist *et al.* 1991). Mercury emissions to the environment, both natural and anthropogenic are dominated by losses of vapour forms to the atmosphere (Andren & Nriagu 1979). These forms have a relatively long residence time (0.7-2 years) conducive to long range transport (Lindqvist *et al.* 1991), which makes the atmosphere the dominant pathway delivering mercury to the world's marine and terrestrial ecosystems (Lindberg 1987, EPMAP 1994). The atmosphere and the oceans are in rapid equilibrium and air-sea exchange processes play a major role in the global environmental cycling of mercury (Fitzgerald 1989). Biologically-mediated

reduction of reactive mercury species to gaseous mercury (Hg^0) takes place in the mixed-layer of the oceans (Mason *et al.* 1994b), while oxidation of Hg^0 to water soluble divalent mercury (Hg^{2+}) in the upper atmosphere is caused by ultraviolet radiation or by interaction with free radicals of ozone (Lindqvist *et al.* 1991). Evasion of Hg^0 from the oceans is balanced by the total oceanic wet deposition of Hg^{2+} from the atmosphere (Mason *et al.* 1994a, EPMAP 1994). Currently the ocean receives about 90% of its mercury through wet and dry atmospheric deposition and contributes over two thirds of the total present natural emissions (Fitzgerald 1989). Such a major role of oceans in the mercury cycle is further illustrated by a three-fold increase in mercury deposition over Antarctica during the last glacial period in relation to variations in marine productivity (Vandal *et al.* 1993). Removal of mercury (in particulate forms) from the mixed-layer into the ocean interior is a poorly understood sink for global atmospheric mercury (Hudson *et al.* 1995). Besides, a small portion of atmospheric mercury (<5%) over continental regions is in the particulate form (Lindqvist *et al.* 1991). The mode of deposition of the particulate fraction differs strongly from that of Hg^0, being dominated by dry deposition and precipitation scavenging. Particulates usually have relatively short residence times and their deposition often shows local- or regional-scale spatial patterns (Lindqvist *et al.* 1991, Nater & Grigal 1992).

The current anthropogenic emissions of mercury to the atmosphere (chiefly from combustion of fossil fuels and waste incineration) exceed direct releases to surface waters by more than an order of magnitude (Andren & Nriagu 1979) and are now considered to exceed the atmospheric emissions from natural processes (Lindqvist 1994, Mason *et al.* 1994a, EPMAP 1994, Hudson *et al.* 1995). Despite human-induced mobilisation of mercury into the biosphere having increased by two or three times between 1900 and 1970 (Andren & Nriagu 1979) there has been considerable uncertainty about the impact of human direct emissions into the global atmospheric load of mercury (e.g. Lindberg 1987, Fitzgerald 1995). Mass balance and model-based analysis (Lindqvist *et al.* 1991, Mason *et al.* 1994a, Hudson *et al.* 1995) suggest a significant influence of anthropogenic atmospheric emissions on the global mercury cycle. That is empirically substantiated by a study of Slemr & Langer (1992) showing a global background increase of atmospheric mercury by over 1% per year in the period 1977-90. In addition, there is evidence of widespread regional historical increases in mercury contamination. Mercury enrichment in lake sediments and peat bog suggests that atmospheric mercury deposition has increased in North America by a factor of 3 to 5 times since the

industrial revolution (Rada *et al.* 1989, Swain *et al.* 1992, Benoit *et al.* 1994) and similar increases were indicated by levels in seabird populations in the North-east Atlantic (Thompson *et al.* 1992). Furthermore, significant effects on the global mercury cycle may arise from potential synergism or antagonism with global changes. Upwelling intensification due to global warming (Bakun 1990) and changes in the general chemistry of the atmosphere (e.g. enrichment in oxidants; Lindberg 1987) can, somehow, affect residence times for mercury in the atmosphere, atmospheric deposition and oceanic evasion.

1.3. MERCURY IN THE STUDY AREA

The Portuguese Atlantic covers a wide oceanic region from the Mid-north to the North-east Atlantic. It comprises an extensive sub-tropical sub-region from the Azores southwards to the Madeiran and Salvages archipelagos, and a temperate sub-region off the coast of mainland Portugal.

Information on mercury contamination of the marine environment in the whole region is very scarce. Regarding the mainland sub-region, industrial discharges to the Tejo estuary have resulted in important contamination in the ecosystem (Ferreira & Oliveira 1988), but information on contamination levels in other areas is not found in the literature. The sub-tropical Portuguese Atlantic is remote from continental anthropogenic emissions of mercury, which suggests that environmental levels of mercury there would represent background levels for mid-latitudes in the North Atlantic. However, mercury levels in marine biota in that sub-region, although lower than in the Mediterranean (Renzoni *et al.* 1986, Monteiro *et al.* 1991), are comparable to levels found throughout 'non-polluted' areas of the Atlantic (Monteiro & Lopes 1990, Monteiro *et al.* 1992, 1995). A tendency for elevated mercury concentrations in waters of the sub-tropical north Atlantic and other sea-water masses underlying jet streams world-wide (Gardner 1975) may be questionable on grounds of sampling and analytical limitations in mercury determinations on seawater prior to the mid-1980s (Gill & Fitzgerald 1985). The prevalence of those environmental levels of mercury in such a remote area might be accounted by some, or a combination of, factors like deposition from air masses transported by the prevailing western winds, long-range transport by jet stream, local volcanism (erupting volcanoes and geothermal activity) and the presence of out flowing Mediterranean water at depths below 1000 m.

Considering the present evidence of global anthropogenic-derived mercury contamination (Slemr & Langer 1992, Mason *et al.* 1994a, Hudson *et al.* 1995), the spatial and temporal monitoring of mercury in the Portuguese Atlantic is advisable and might provide valuable insights into local-, regional- and global-scale processes on cycling of mercury.

1.4. SEABIRDS AS MONITORS FOR MERCURY

Mercury monitoring in marine ecosystems may rely upon the quantification of mercury in abiotic (air, water, sediment) and biotic (living organisms) compartments. Monitoring may be designed in relation to a variety of objectives: to measure the level of environmental contamination or the rate of change in contamination, to assess the rate of release into the environment, to assess the biological effects on species or communities, or to assess the hazard to humans. A programme that is optimally designed for one purpose if often unsuitable for others.

Because mercury cycling comprises many biological-mediated processes, biomonitors offer particular advantages to quantify mercury abundance and availability in aquatic ecosystems (Phillips 1980). Seabirds, in particular, offer a number of advantages as monitors of mercury contamination in the marine environment (reviews in Walsh 1990, Furness 1993, Monteiro & Furness 1995). They are numerous, colonial, and the ecology of most species is well known; they are top predators and thus will integrate contamination over food webs, reflecting slight variations in environmental mercury; seabird foraging ranges vary from restricted to wide and, according to species and time relative to the annual cycle, they offer varying spatial and temporal levels of integration of mercury contamination, from coastal to oceanic environments.

The current knowledge on the dynamics of mercury in birds gives a good basis for the use of seabird as monitors of mercury. Nevertheless, there is still considerable scope for an experimental appraisal of methylmercury kinetics and dose-responses in free-living birds. It is particularly relevant to fill the lack of knowledge on the kinetics of methylmercury in avian blood, due to its role as internal carrier and ubiquous contact with all other tissues.

Internal tissues, blood, eggs, feathers and chicks have been widely used as monitoring units. Feathers are the most attractive amongst them. They are both

chemically and physically stable (Crewther *et al.* 1965, Appelquist *et al.* 1984), generally accumulate higher mercury levels than other tissues (Monteiro & Furness 1995), levels are not affected by atmospheric deposition (Hahn *et al.* 1993) and their sampling is non-destructive. In addition, feathers from birds in museum collections offer a great potential for the study of synoptic geographical and historical changes in mercury contamination (e.g. Thompson *et al.* 1992). Mercury concentrations in eggs and body tissues have been analysed frequently since the mid 1960s and there is a growing database of mercury levels in seabirds populations, particularly in the northern hemisphere. However, to establish accurate monitoring studies of mercury using seabirds, besides an adequate combination of species and monitoring units, it is important to quantify unwanted intra-specific sources of variation in order to distinguish between background noise and signals due to environmental variation.

1.5. AIMS AND STRUCTURE OF THE THESIS

This study aims to provide further insights into the dynamics of mercury in birds and realise a first comprehensive assessment of the mercury contamination in the Portuguese Atlantic using seabirds as monitors. It includes a general literature review and field studies on the ecology of the seabird assemblage (Chapters 2, 5 and 6) and a review on the mercury dynamics in seabirds and their use as monitors (Chapter 3). Then, a non-destructive multi-tissue approach, using blood, plumage and eggs, is employed to:

1. Quantify the sources of intra-specific variability of mercury levels in potential monitor species (Chapter 7);
2. Investigate the role of feeding ecology on seabird's mercury burdens (Chapter 8);
3. Establish the role of blood on mercury dynamics in adult and young seabirds (Chapters 7, 9 and 10) and derive relationships between steady-state dietary intake of methylmercury and blood concentrations (Chapters 9 and 10);
4. Experimentally evaluate kinetics, dose-response relationships and excretion of methylmercury in free-living adult and young seabirds (Chapters 9 and 10);
5. Assess contemporary spatial patterns of mercury contamination in the Portuguese Atlantic (Sub-chapter 11.1);

6. Assess historical trends of mercury contamination in the ecosystem of the study area (Sub-chapter 11.2).

The reader's attention is drawn to the format of this thesis. Each chapter or sub-chapter was prepared in a form appropriate for publication as independent papers and therefore has its own introduction, methods, results and discussion sections, and reference list. At submission of this thesis (January 1996) some chapters were published and others were submitted for publication. These were adapted to the general layout of the thesis but spelling, citations and reference list were kept in the original formats.

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CHAPTER 2

A REVIEW AND OBSERVATIONS ON SEABIRDS BREEDING IN THE AZORES

CONTENTS

CHAPTER 4

MATERIALS AND METHODS

CONTENTS

4.1. STUDY AREA AND SPECIES

The Portuguese Atlantic covers a wide sub-tropical and temperate area from the Mid-north to the North-east Atlantic. The study area extended from the Azores to the coast of mainland Portugal and then southwards to the Madeiran and Salvages archipelagos (see Fig. 11.1.1).

The species used in this study comprise eight of the fifteen regular breeders in the Portuguese Atlantic: five Procellariiformes -Bulwer's petrel *Bulweria bulwerii*, Cory's shearwater, Little shearwater *Puffinus assimilis baroli*, Madeiran storm petrel *Oceanodroma castro*, White-faced storm petrel *Pelagodroma marina hypoleuca*-, plus three Laridae -Yellow-legged gull *Larus cachinnans atlantis*, Common tern *Sterna hirundo*, Roseate tern *Sterna dougallii*.

The work was developed conforming to current guidelines for use of wild birds in research (Lewis *et al.* 1988) and under appropriate licenses from the relevant authorities throughout the study area.

4.2. SAMPLE COLLECTION

4.2.1. General

The choice of seabird tissues as monitoring units for mercury was based on recent reviews (Furness 1993; Monteiro & Furness 1995) and non-destructive sampling procedures were adopted. Birds were mist-netted or captured by hand on the ground or in burrows, except for adult terns, which were captured using nest traps. Sampling periods, specific biological information and collection details are given in the relevant chapters.

4.2.2. Eggs

Abandoned and incubated eggs give the same indication of mercury levels in a population (Ohlendorf *et al.* 1988, Becker *et al.* 1993). Thus, for ethical reasons, only abandoned eggs were collected for Procellariiformes and terns while for the gull an incubated egg per clutch was collected at random. Eggs with broken shell were rejected due to potential loss of contents. Eggs were stored deep frozen at

ca. -20°C prior to further treatment. Specific collection details for particular samples obtained in this way are given in the relevant chapters.

4.2.3. PLUMAGE

4.2.3.1. Contemporary samples

Down and contour feathers have been taken preferentially, except when otherwise outlined in the relevant chapters. Because differences between the two coats of down of Procellariiformes chicks may be subtle at certain developmental stages, samples of primary down were collected within two weeks of hatching and samples of secondary down were collected when development of contour feathers was advanced. Samples of 2-10 feathers, depending on the relative size of contour feather types, were plucked from each individual adult or well grown chicks and stored in polyethylene bags; equivalent masses of down were plucked from small chicks and stored in polyethylene bags. Designation of feather types followed Brooke & Birkhead (1991).

Plumage samples were obtained at seabird colonies from live, apparently healthy birds during the breeding season, except for about two-thirds of the breast feather samples of adult Yellow-legged gull from Azorean colonies, which were collected from corpses. Because moult is a main confounding factor for feather mercury concentrations (Furness *et al.* 1986), information on moult status of each individual sampled was recorded. Special care was taken to control for feather's age when the same individuals were sampled repeatedly over the breeding seasons. This was achieved using standard sampling procedures (i.e. alternate lateral sampling in the ventral region) and consulting updated data bases in the field, or marking feathers with picric dye in cases of prolonged sampling periods. It was found that ventral feathers take more than one month to complete growth in Procellariiformes, attaining a maximum length of ca. 4 cm in Cory's shearwater over that period (unpubl. data).

4.2.3.2. Historical samples

Historical feather samples were obtained from preserved study skins in museum collections. Breast and flank feathers, also axillaries in a few cases, were taken from skins of adults with known date of capture and were stored in polyethylene bags until analysis.

4.2.4. Blood

Collection of blood samples from wing and leg veins does not impair behavioural patterns, reproduction and survival of wild birds (Lewis *et al.* 1988). In this study, blood samples from live, apparently healthy birds, were collected from vessels in the tibio-tarsi for larger birds (above 100 g) and from vessels in the foot web for smaller birds (below 100 g), using sterilised disposable syringes with 0.5x16 mm 25G or 0.36x13 mm 28G needles. Volumes taken varied from 0.2 to 2 ml and were below the lowest of the recommended maximum proportion of 10-20% of total blood volume (Lewis *et al.* 1988), based on a blood volume of 6-8 ml per 100 g body mass (Sturkie 1986).

The samples were placed in heparinised polypropylene tubes and, within a week of collection, were frozen at ca. -20°C until further treatment. Short-term storage of blood samples at ca. 20°C or storage in a refrigerator for several months does not cause losses of mercury from blood samples (Sällsten *et al.* 1993).

4.2.5. Fish and food samples

Fish species likely to be preyed upon by seabirds were collected for total mercury analysis. Specimens were obtained from the fish collection at the University of the Azores (Department of Oceanography and Fisheries), fishing boats and fish dropped at seabird colonies, and covered a wide range of vertical distributions, from epipelagic to mesopelagic environments. Specific details on sampling periods, fish size and methods of preservation are presented in the relevant chapter (Sub-chapter 8.1).

Food samples, consisting of spontaneous or induced regurgitations and pellets (only for Common tern), were collected for study of diet composition and determination of total mercury concentrations. Specific details on sampling periods, methods of preservation and identification of dietary items are presented in the relevant chapter (Sub-chapter 8.2).

4.3. SAMPLE PREPARATION

4.3.1. FEATHERS

Some studies report that efforts to remove surface contaminants prior to analysis can affect the mercury content of human hair while others observed little or no effect (review in Airey 1983). In this study, the effect of washing on feather mercury concentrations was assessed by subjecting duplicates of 4 different samples (each made of 12 or more breast feathers) to 2 distinct washing regimes (Table 4.1). In regime 1, feather sub-samples were subjected to a chloroform/acetone laundering described in detail by Muirhead (1986). In regime 2, feather sub-samples were washed with 5% neutral detergent Extran in an ultrasonic bath for 10 min, then washed 4 times with distilled water in the ultrasonic bath, dried at 50°C in an oven for 24 h and allowed to equilibrate with ambient laboratory temperature (ca. 20°C) prior to weighing; unwashed sub-samples were used as controls. Mean mercury concentrations in unwashed samples were almost identical to those in washed samples (Table 4.1). This indicated absence of measurable surface contamination with mercury as reported in other studies (Airy 1983, Lewis 1991). Thus, only a few feather samples with major dust-surface contamination, likely to alter feather weight, were subjected to washing regime 1.

Growing feathers with plasma and/or blood in the calamus were preserved by dehydration at 50°C for up to 48 h in an oven and then allowed to equilibrate with ambient laboratory temperature (ca. 20°C) for 48 h prior to storage or weighing.

Because consistent weights of dried oven feather samples were difficult to obtain due to rapid reabsorption of moisture (Thompson 1989), feather weights were taken as those obtained at ambient laboratory temperature and therefore are expressed on a fresh weight basis.

Contour feathers were analysed whole, but primaries and rectrices were homogenised in a food cutter and sub-samples were taken for mercury analysis.

Table 4.1. - Effect of washing regime on mean mercury concentrations (μg/g, fw) in duplicates of 4 different feather samples.

Washing	Sample			
regime	A	B	C	D
unwashed	0.8	1.1	3.6	5.7
1	0.9	1.5	6.6	5.5
2	1.6	1.0	3.6	5.8

4.3.2. EGG, BLOOD, FISH AND FOOD SAMPLES

Preparation of egg, blood, fish and food samples for mercury analysis involved dehydration in an oven at 50°C to a constant weight to avoid bias in mercury concentrations when using wet weights (Adrian & Stevens 1979). Other particular matrix preparation procedures were:

1. Each egg was thawed and opened, the whole internal content (yolk plus albumen) was removed; in some cases the yolk and albumen were separated. The whole or separated internal contents were then dried, homogenised using a food cutter and stored in air-tight glass or polystyrene containers until analysis.
2. Whole blood samples were thawed, dried and then ground using a pestle and mortar and stored in air-tight glass or polystyrene containers until analysis.
3. Whole fish and food samples were thawed or taken from preservative solution, as appropriate, and dried. They were then ground using a pestle and mortar and stored in an air-tight glass or polystyrene containers until analysis.

In order to permit accurate conversion of data to a wet weight basis, mean moisture content for various tissues and species are provided in Table 4.2. Percent moisture in whole egg contents and whole fish varied in a highly significant way among species (1-way ANOVAs, respectively, $F_{5,97} = 27.89$ and $F_{4,79} = 13.76$, $P < 0.0001$ in both cases). Moisture in adult and chick blood was identical; moisture in albumen was identical among species (overall mean = 86.1%), while in yolk it showed a tendency to higher values in Laridae (mean = 57.9%) than in Procellariiformes (mean = 52.1%).

Table 4.2. - Percent moisture content in various types of biological samples and species.

Sample type	Species	Mean [a]	S.E.	n
Whole egg	Bulwer's petrel	69.0[A]	0.8	12
contents	Cory's shearwater	72.9[B]	0.5	23
	Madeiran storm petrel	69.9[A]	0.4	13
	Yellow-legged gull	75.8[C]	0.3	27
	Common tern	74.5[B,C]	0.4	17
	Roseate tern	74.2[B,C]	0.6	11
Yolk	Bulwer's petrel	51.7	-	1
	Cory's shearwater	51.7	0.4	9
	Common tern	56.8	-	2
	Yellow-legged gull	59.1	-	2
Albumen	Bulwer's petrel	85.7	-	1
	Cory's shearwater	86.8	0.3	9
	Common tern	86.3	-	2
	Yellow-legged gull	86.0	-	2
Whole blood	Cory's shearwater, adults	79.6	0.3	10
	Cory's shearwater, chicks	79.7	0.5	16
Whole fish	*Macroramphosus scolopax*	65.7[A]	1.5	41
	Capros aper	66.9[A]	0.7	14
	Maurolicus muelleri	71.2[B]	0.6	14
	Ceratoscopelus maderensis	73.7[B]	0.8	12
	Myctophum punctatum	75.0[B]	1.5	3

[a] Within sample type, means with different upper case superscripts are significantly different (Tukey tests, $P<0.05$)

4.4. MERCURY ANALYSIS

4.4.1. TOTAL MERCURY ANALYSIS

4.4.1.1. Principle of the method

Total mercury was determined by cold vapour atomic absorption spectrophotometry (CVAAS, Hatch & Ott 1968). This is a physical method based on the absorption of radiation at 253.7 nm by mercury vapour. The mercury is reduced to the elemental state and aerated from solution in a closed system. The mercury vapour passes through a cell positioned in the light path of an atomic absorption spectrophotometer and absorbance (peak height) is measured as a function of mercury concentration.

4.4.1.2. Reagents

Analytical grade reagents and distilled water were employed and solutions were prepared in the following manner:

Stock mercury solution (i), 1000 mg/l - Dissolve 0.1354 g of mercuric chloride in 75 ml of distilled water. Add 10 ml of conc. nitric acid (sp. gr. 1.40, $Hg < 5x10^{-7}$ %) and adjust to 100 ml.

Working mercury solution (ii), 1 mg/l - Made from successive dilutions of the stock mercury solution. This working standard solution and the dilutions of the stock mercury solution were prepared daily, with acidity maintained at 0.15% nitric acid. The acid should be added to the flask as needed before the addition of the aliquot.

Sulphuric acid (sp. gr. 1.84, $Hg < 5x10^{-7}$ %).

Potassium permanganate solution, 6% w/v - Dissolve 120 g of potassium permanganate in 2000 ml of water, using a heated magnetic stirrer for 1 hour, and store in a brown glass bottle.

Hydroxylammonium chloride solution, 20% w/v - Dissolve 100 g of mercury-free ($Hg < 1x10^{-6}$ %) hydroxylammonium chloride in 500 ml of water.

Stannous chloride solution, 10% w/v - Dissolve 25 g of stannous chloride dihydrate in 50 ml of hydrochloric acid (sp. gr. 1.18) and dilute to 250 ml. Heat the resulting solution with two 5x50 mm tin foils and boil for 30 seconds. Store over tin in a dark glass bottle for a maximum of a week.

4.4.1.3. Preparation of the sample solutions

Samples were subjected to a wet mineralisation digestion prior to mercury determination. The procedure employed was adapted from Anon. (1975) and is described below. Each batch was made of up to ca. 60 samples and analysed over two days.

On day 1, sub-samples of 0.01-2 g were placed in BOD (or Winkler) flasks and weighed accurately (to 0.001 g) using an electronic top pan balance. Then 10 ml of sulphuric acid was added to each sample and the flasks were placed in a water bath at 70°C for 6 h. Flasks were shaken regularly during the first two hours to aid sample mineralisation. Flasks were left overnight at room temperature.

On day 2, digestion was completed by adding 50 ml of 6% potassium permanganate to cooled flasks and placing them in a water bath at 70°C for 2 h. The flasks were cooled and the excess of potassium permanganate was reduced by adding 15 ml of hydroxilamine hydrochloride solution.

4.4.1.4. Determination

Mercury determinations were made with Perkin-Elmer Mercury Analysers Coleman 50A or 50B. A desiccant cell, with magnesium perchlorate, was installed between the aerator and the absorption cell. The apparatus was switched on at least 2 hours before use and the procedure employed for determination of mercury in samples is described below.

To achieve a convenient distribution of the liquid and gaseous phases, 50 ml of distilled water were added to each BOD flask. Subsequently, divalent mercury was reduced to elemental mercury by adding 2 ml of the stannous chloride solution into the BOD flask. The aerator of the apparatus was immediately attached to the bottle forming a closed system and the sample was agitated manually and gently for ca. 5 seconds. The absorbance increased towards a maximum value which was noted. The aerator was disconnected and aeration continued until the absorbance returned to the baseline.

The analysers used allow a direct reading of the mass of mercury (in µg) in the sample, which was employed in the following equation to calculate the mercury concentration (Hg, µg/g or ppm) in the sample (mass in grams),

$$Hg = \frac{reading - blank}{mass} \qquad (4.1)$$

4.4.1.5. Quality control

The limit of detection of the method, taken as twice the standard deviation of triplicate analysis at blank concentrations (Saltzman *et al.* 1983), was 10 ng, equivalent to 0.01 μg/g for a 1 g sample. Within- and between-laboratory quality control procedures were employed throughout the study period.

Accuracy of the method, expressed as relative error (RE = 100*(observed-expected)/expected), was within 10% and monitored throughout the study with standards of inorganic mercury and reference materials with matrix similar to the samples: NIES human hair RM n°5, NRCC dogfish muscle DORM 1 (Table 4.3). During the study, the laboratory also participated in the intercomparison program for mercury in human hair undertaken by the National Research Council of Canada (NRCC).

Interference on sensitivity due to matrix and pre-treatment were assessed by the method of standard additions before the wet mineralisation digestion. Recoveries of added inorganic mercury varied significantly among matrix types (Table 4.4; 1-way ANOVA, $F_{3,48} = 7.27$, $P < 0.0005$). The mean recovery differed significantly from 100% for whole fish and regurgitations and concentrations were corrected accordingly in the relevant chapters.

Precision (or reproducibility) of the method, expressed as coefficient of variation (CV = 100*S.D./mean) of duplicates within batch, varied significantly among matrix types (Table 4.5; Kruskal-Wallis test, $H_{4,n=130} = 16.89$, $P < 0.01$). The coefficient of variation was highest in feathers and blood and lowest in down, which approached the variability attributable to the technique observed from standard solution samples. The coefficients of variation of duplicate determinations of mercury (mean $\pm$S.E., μg/g fw) in breast feather samples (normally made of 2-3 feathers) from a variety of species did not differ significantly within-batch (10.4$\pm$1.9, n = 28; from Table 4.5) or between-batch (10.7$\pm$1.0, n = 88; $t_{114} = 0.12$, $P = 0.90$). In addition, the coefficient of variation was found to be independent of the mercury concentration in the feather sample, over a wide range of levels (0.1 to 30 ppm fw, Fig. 4.1; Product-moment correlation, $r = 0.14$, $P = 0.14$, $n = 110$). In conclusion, the precision of the method was within the usual 10% for total mercury determinations in biological samples (Dehairs *et al.* 1982, Saltzman *et al.* 1983).

Table 4.3. - Observed and expected total mercury concentrations (µg/g, dw) and relative error for analysed standard reference materials.

Reference material	Observed[a]	Expected[b]	RE (%)
NRCC, DORM 1	0.829 ±0.038 (9)	0.798 ±0.74	+3.9
NIES, RM 5	4.3 ±0.2 (12)	4.4 ±0.4	-2.3

[a] mean ±S.D., sample size bracketed.
[b] mean ±95% confidence limits.

Table 4.4. - Recovery of standard additions of inorganic mercury (mean ±S.E., sample size bracketed, range below) in samples of different matrix.

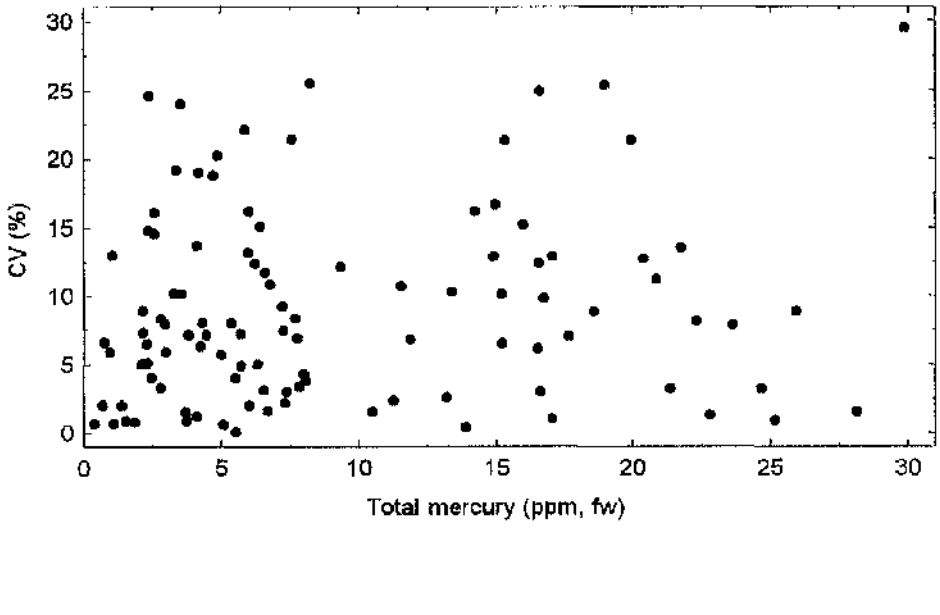
Matrix	Recovery (%)	t-test [a]
egg	93.3 ±4.5 (16) 70-133	$t_{15} = 1.50^{NS}$
feather	99.8 ±5.6 (8) 79-131	$t_7 = 0.044^{NS}$
whole fish	79.2 ±2.7 (18) 67-103	$t_{17} = 7.61^{***}$
regurgitations	69.3 ±6.7 (8) 50-108	$t_7 = 4.58^{**}$

[a] two-tailed t-test for difference between the mean and a hypothesised mean of 100%; NS - $P > 0.05$, ** - $P < 0.005$, *** - $P < 0.0001$.

Table 4.5. - Coefficient of variation for within batch duplicate determinations of total mercury in standard solutions and seabird tissues.

Matrix	Mean	S.E.	n
$HgCl_2$ solution	4.6	0.4	44
egg	8.5	1.0	27
feather	10.4	1.9	28
down	5.7	0.9	16
blood	10.2	1.6	15

Fig. 4.1
Scatterplot of coefficients of variation from mercury determinations in duplicates of feather samples and total mercury levels in those samples.



4.4.2. Organic mercury analysis

4.4.2.1. Scope and principle of the method

All historical feather samples were analysed for organic mercury instead of total mercury to overcome potential problems of inorganic mercury contamination from preservatives in museum skins (Thompson & Furness 1989).

Organic mercury was extracted from samples following the method developed by Uthe *et al.* (1972) and adapted by Thompson & Furness (1989). The method did not permit speciation of organo-mercurials but, since monomethylmercury is the form normally found in biological samples (Horvat *et al.* 1988), it was assumed that organic mercury extracted was monomethylmercury. The procedure involves extracting methylmercury into toluene, as methylmercuric bromide, followed by stripping of methylmercury from toluene into thiosulphate. Mercury in the extracted samples was then determined by CVAAS.

4.4.2.2. Reagents

Analytical grade reagents and distilled water were employed and solutions were prepared in the following manner:

Stock methylmercury solution (i), 1000 mg/l - Dissolve 0.1252 g of methylmercuric chloride in 75 ml of distilled water and adjust to 100 ml.

Working methylmercury solution (ii), 1 mg/l - Made from successive dilutions of the stock methylmercury solution. This working standard solution and the dilutions of the stock methylmercury solution were prepared daily, with acidity maintained at 0.15% nitric acid. This acid should be added to the flask as needed before the addition of the aliquot.

Sodium hydroxide solution, 10M - Dissolve 40 g of sodium hydroxide in 100 ml of water, cool and store in a polypropylene bottle.

Sulphuric acid (sp. gr. 1.84, $Hg < 5x10^{-7}$ %).

Acidic sodium bromide solution - Dissolve 250 g of sodium bromide in 565 ml of water, to which was added 89 ml of conc. sulphuric acid/89 ml of water, and store in a brown glass bottle.

Copper sulphate solution, 0.1M - Dissolve 25 g of copper sulphate pentahydrate in 1000 ml of water and store in a brown glass bottle.

Toluene (sp. gr. 0.87).

Stock sodium thiosulphate solution (i), 0.05M - Dissolve 12.4 g of sodium thiosulphate pentahydrate in 1000 ml of water and store in a brown glass bottle.

Working sodium thiosulphate solution (ii), 0.005M - Made from dilution of the stock sodium thiosulphate solution. This working solution was prepared daily.

4.4.2.3. Extraction and determination

Samples of ca. 0.04-2 g were placed in 50 ml Kjeldahl flasks with stopper and weighed accurately (to 0.001 g) using an electronic top pan balance. Then, 4 ml of sodium hydroxide was added to the sample and the flask was placed in a water bath at 50°C for 3 h. Flasks were shaken regularly at this stage to aid the sample to be broken down. The sodium hydroxide solution was subsequently neutralised using 0.85 ml conc. sulphuric acid.

Samples treated in the above way were transferred to a 50 ml centrifuge tube, added 10 ml of copper sulphate, 5 ml of acidic sodium bromide and 10 ml of toluene, and then thoroughly mixed in a vortex for 2 min. Mixed samples then underwent a centrifugation for 15 min at 4000 rpm and 5 ml of the toluene was removed, using a graduate glass syringe, to a 15 ml centrifuge tube.

The 5 ml of toluene was thoroughly mixed with 2 ml of the working sodium thiosulphate solution in a vortex for 1 min. The toluene/sodium thiosulphate mixture underwent a centrifugation at 2000 rpm for 10 min and 1 ml of the aqueous phase was removed using a graduated syringe and placed in a BOD flask. The flask was placed in a water bath at 50°C for 1 h to drive off any residual

toluene and was left overnight at room temperature. Samples were mineralised and digested the following day as described in section 4.4.1.3, but with just 1 h of digestion in sulphuric acid at 70°C, and mercury was determined as described in section 4.4.1.4.

4.4.2.4. Quality control and calculations

The extraction method efficiency was tested by performing extractions of standard solutions of organic mercury. The efficiency of triplicate extractions of 1 µg of mercury as methylmercuric chloride increased significantly with increasing time of mixing: 0.823±0.019 S.E. for 0.5/0.5 min to 0.910±0.020 S.E. for 2/1 min in the vortex ($t_4 = 3.16$, $P < 0.05$). This served to set the standard times of mixture in the vortex employed in the extraction procedure (section 4.3.2.3). In addition, the efficiency of extraction was monitored throughout the study with replicates of samples with 1 µg of mercury as methylmercuric chloride, in two ways: (1) comparison of expected and observed levels of total mercury in extracted samples; (2) comparison of total mercury levels determined in extracted samples with levels determined in non-extracted samples (Table 4.6). The resulting estimates of extraction efficiency did not differ significantly between the two methods ($t_{10} = 0.24$, $P = 0.82$), producing an overall mean of 0.910. All methylmercury levels obtained in subsequent analysis were corrected accordingly. This extraction efficiency is in close agreement with that of 0.900 determined by Thompson & Furness (1989). In addition, Thompson & Furness (1989) demonstrated that the extraction efficiency was independent of the methylmercury levels extracted, that inorganic mercury was not extracted by the method and that matrix effects were absent when analysing feather samples or horse kidney reference material.

Calculation of methylmercury concentrations accounted for the fact that the 1 ml extracted sample effectively contained 25% of the methylmercury originally present in the sample and for the efficiency of the extraction, and followed the equation,

$$MeHg = \frac{reading - blank}{mass * 0.25 * 0.910} \qquad (4.2)$$

Accuracy and precision of organic mercury determinations performed here (expressed as relative error and coefficient of variation, respectively) were within 10% and were monitored throughout the study with standard reference materials

and standards of methylmercuric chloride. The mean organic mercury in NRCC dogfish muscle DORM 1 was 0.770 μg/g $\pm$0.040 S.E. (n=3, CV=8.9%) compared with the certified value of 0.731 ($\pm$95% confidence interval=0.060); the mean organic mercury in IAEA tuna fish reference material 350 was 3.90 μg/g $\pm$0.14 S.E. (n=3, CV=6.2%) compared with a value of 3.96 μg/g $\pm$0.12 S.E. (n=5) given by Horvat (1991). The mean coefficient of variation for replicate standard solution samples with 0.5-1 μg of mercury as methylmercuric chloride was 4.1 % $\pm$1.0 S.E. (n=9).

Table 4.6. - Organic mercury extraction efficiency (mean $\pm$S.E., sample size bracketed) in replicate samples of 1 µg of mercury as methylmercuric chloride, during the study period.

Method	n	Mean	S.E.	Range
1	7	0.912	0.014	0.846-0.960
2	5	0.907	0.013	0.879-0.938
overall for both methods	12	0.910	0.009	0.846-0.960

4.4.3. GLASSWARE LAUNDERING

Glassware was cleaned by rinsing with tap water and then washing in a machine (Miele, Mielabor G7783) supplied with water from a Miele Aqua Purificator G7794 and Neodisher A8 detergent.

4.5. STATISTICAL ANALYSIS

Data were tested for goodness of fit to a normal distribution using the Kolmogorov-Smirnov one-sample test and the requirements of homogeneity of variances using the Levene test, prior to statistical analysis. Parametric and non-parametric statistics were employed as appropriate, and analysis followed standard statistical procedures (Zar 1984, Tabachnick & Fidell 1989) with casewise deletion of missing data.

Pooled data from various breeding seasons were used and statistical tests of differences among years were not performed because data were not fully independent (e.g., many of the birds nested in the colony in successive years). Other specific details relating to tests used are presented in the individual chapters. All statistical tests were considered significant when $P < 0.05$.

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CHAPTER 5

SPECIATION THROUGH TEMPORAL SEGREGATION OF BAND-RUMPED STORM PETREL (*Oceanodroma castro*) POPULATIONS IN THE AZORES?

CONTENTS

5.1. INTRODUCTION

The Band-rumped storm petrel *Oceanodroma castro* is widespread in subtropical areas of the Pacific and Atlantic Oceans. The known breeding distribution comprises sites off the east coast of Japan, in Hawaii and Galapagos, off the west coast of Portugal, in Madeira, Salvages, Canary Is., Cape Verde, Ascension and St. Helena (Allan 1962, Banko *et al.* 1991, Harris 1969, Martin *et al.* 1984, Teixeira and Moore 1981). There may be a separate subspecies breeding on São Tomé, in the Gulf of Guinea (Harris 1969, Williams 1984). Although the species has long been known to occur in the Azores (Hartert and Ogilvie-Grant 1905), there was no proof of breeding (Bannerman and Bannerman 1966, Cramp 1977) until recently (Monteiro *et al.* 1996a,b).

The Band-rumped storm petrel shows geographical variation, with several subspecies described based on differences in bill structure, wing length and amount of white on the rump. However, the validity of these subspecies is unclear (Austin 1952) and the species has been treated as monotypic (Cramp 1977, Jouanin and Mougin 1979, Warham 1990).

The breeding cycle of this species shows a noteworthy plasticity across its range, described as two seasons a year in Galapagos (Snow and Snow 1966), one season with some out-of-season nesting in Ascension (Allan 1962) and an extended season spread over the whole year in the Salvages (Mougin *et al.* 1990). However, only the populations in Ascension and Galapagos have been studied in detail and phenologies in other areas are unclear. Two temporally segregated populations breed in Galapagos (Harris 1969). On the basis of significant differences in mercury concentrations in the plumage between hot season (Spring) and cool season (Autumn) breeders in the Azores, Monteiro *et al.* (1995) suggested that the Band-rumped storm petrel there may comprise two discrete populations. The hypothesis is supported by recent information on its distribution (Monteiro *et al.* 1996a) and phenology (Monteiro *et al.* 1996b).

In this paper we present a comprehensive analysis of data on breeding phenology, molt, segregation of adults between populations and morphology of hot and cool season breeders in the Azores, and we discuss the possible ecological adaptations underlying the observed temporal partitioning of reproduction as well its taxonomic implications.

5.2. STUDY AREAS AND METHODS

The study areas comprised all known colonies of the species in the Azores (36-39°N, 25-31°W), situated in three small rat-free islets (Monteiro *et al.* 1996a): Vila (off Santa Maria) and Baixo and Praia (off Graciosa and 4 km apart); Santa Maria and Graciosa are about 300 km apart. The data were obtained during monthly visits of 2 to 10 days to Vila, between April-December 1993, March-October 1994 and January, March 1995, and (when sea conditions permitted) to Praia and/or Baixo between April-November 1993, in March, June and August 1994 and in March 1995. Each islet was explored thoroughly to determine the distribution, numbers and status of storm petrel nest sites. The topography and geology of each islet dramatically affected the number of accessible active nest sites that we found, which totaled 131 at Vila, 20 at Baixo and 5 at Praia. The breeding populations were estimated as 600 pairs in the cool season plus 200 pairs in the hot season (Monteiro *et al.* 1996a).

Most adults were captured in mist-nets and a few were removed from burrows; playback was not used. Adults and chicks were individually banded with numbered metal bands. Masses and up to nine mensural characters were taken by the first author: NAP-nape (head+bill), CUL-culmen (bill length), NOS-nostrils (bill depth at), GON-gonys (bill depth at), TAR-tarsus, WIN-wing (flattened chord), TMA-tail maximum (dorsally from uropygial gland to tip of longest rectrix), TMI-tail minimum (dorsally from uropygial gland to tip of shortest rectrix) and MAS-body mass; FOR-depth of tail fork was calculated (difference between tail maximum and tail minimum). Egg length, breadth and mass were measured. Linear measurements were taken with Vernier/dial calipers (to 0.1 mm) or with a metal ruler (to 1 mm). Egg, chick and adult masses were taken with 50g (eggs and small chicks) and 200g Pesola scales (to 0.5 and 2 g, respectively). The state of the brood patch was scored: 0 (no down was shed), 1 (only traces were lost), 2 (about half of the patch was down covered), 3 (traces of down remained), 4 (entire brood patch free of down) (Furness and Baillie 1981) and R (refeathering present). Molt of breast plumage was recorded as absent or in progress.

Univariate and multivariate statistical analyses followed standard procedures (Zar 1984, Tabachnick and Fidell 1989) with casewise deletion of missing data. One-way analyses of variance (ANOVA) were followed by Tukey-tests for *a posteriori* pairwise comparison of means. Multivariate analyses were used to investigate adult morphology. Principal components were extracted,

reducing the extensive morphological information to a smaller number of mutually independent variables which account for most of the phenotypic variation included in the original set of data. Then, a multivariate analysis of variance (MANOVA) was performed to test differences on principal components among populations. Finally, a stepwise discriminant analysis was used to examine differences between *a posteriori* groups indicated by principal component analysis (PCA) and MANOVA.

The relative difference in means of two sets of measurements (NAP, CUL, NOS, GON and TAR) made by LRM on different occasions on the same 50 birds were lower than 0.29% and not significant (paired t-tests, $p > 0.05$), except for NOS (rel. diff. = 1.78%, $t = 3.78$, $P < 0.001$), indicating an overall consistency of measuring. Brood patch scores of adults in each month did not differ significantly within colonies between years 1993 and 1994 (Mann-Whitney U-tests, $p > 0.05$) and pooled data were used for analysis. Measurements of adults and eggs from 1993 and 1994 also did not differ significantly within colonies (t-tests, $p > 0.05$) and pooled data were also used for analysis.

5.3. RESULTS

5.3.1. BREEDING PHENOLOGY

5.3.1.1. Colony attendance

Adults returned to Baixo and Praia by the end of March and numbers built up over the following weeks. Capture rates of birds in 12m mist-nets were 0.3, 3.2 and 5.0 birds/hour/net on 29 March 1994, 16 April and 17 May 1993, respectively. Adults were recorded in these two colonies in every month from the end of March to mid-November (the latest visit). In contrast, on Vila adults were absent until early August (with one exception, see below) and then numbers built up gradually during September. Capture rates of birds in 12m mist-nets in 1993 were 0.8 and 3.6 birds/hour/net on 14 August and 14 September, respectively. During 44 nights spent on Vila between late March and late July 1993 and 1994, the Band-rumped storm petrel was registered only once, on 18 June 1993. Then a single flying bird was calling repeatedly until an adult was caught and calling ceased. This suggested that the same bird was involved. This was probably a non-breeder (brood patch, BP = 3) and it was caught again on Vila on 28 August 1994 (BP = 4).

5.3.1.2. Brood patch

Brood patch scores of adults did not vary significantly between Baixo and Praia (Mann-Whitney U-tests, $p > 0.05$; June to September), and pooled data from these two colonies were compared with the state of brood patches of adults on Vila (Table 5.1). In September brood patches were less developed in Vila than in Baixo/Praia but in October and November median scores did not differ statistically between the two islands (Mann-Whitney U-tests, $p > 0.05$).

The monthly development of brood patch scores of adults mist-netted during the breeding season is summarized in Table 5.2. Score R, which is indicative of hatching (brood patch starts to regrow a week after hatching and in non-breeders at the same time; Harris, 1969) occurs in two distinct periods on average about four months apart. The monthly frequency distribution of Score 4 is bimodal, the two annual peaks (over 60%) being May-June and October-November, with an interval of about 5 months. Brood scores 0-3 showed a rapid decrease across two main periods, along with increases in scores 4 and R: in April to July and September to December. The distribution of brood scores in August suggests an overlap of hot season birds, represented by scores 4 and R (i.e. 70% of total) with the first returning cool season birds, reflected in the reappearance of score 0 accounting for 20% of the total (see Table 5.2).

5.3.1.3. Egg stage

An insight into laying dates is given by the capture of 8 pre-laying females carrying eggs, during 1993: on Baixo/Praia singles on 17 May, 24 June, 23 and 25 September and three on 25 October; on Vila one on 22 October. A total of 67 eggs in incubation were found in 1993 and 1994 in the following months (three islets pooled): June (16), July (6), September (1, on the 23rd), October (23), November (7), and December (14).

Egg measurements were classified into two groups: hot season (laid in June-July) and cool season (laid in October-December) (Table 5.3). Lengths and breadths of cool season eggs were pooled from Baixo/Praia ($n = 5$) and Vila ($n = 50$), since these did not differ significantly (t-tests, $p > 0.05$). Eggs from cool season birds were significantly longer, heavier and larger than those from hot season birds although egg breadth did not differ significantly between seasons (see Table 5.3).

TABLE 5.1. - Brood patch scores of adult Band-rumped storm petrels mist-netted on Baixo/Praia and Vila. Values are mean and sample size (bracketed) and Z_{adj} values for Mann-Whitney U tests (ns-not significant; ***-$p<0.001$).

Month	Baixo/Praia	Vila	Z_{adj}
September	2.7 (366)	1.7 (122)	6.40^{***}
October	3.6 (41)	3.4 (87)	0.53^{ns}
November[a]	3.9 (33)	3.9 (41)	0.60^{ns}

[a] score R excluded.

TABLE 5.2. - State of brood patches by month in adult Band-rumped storm petrels mist-netted in Azores colonies. Data from Baixo/Praia (April-November 1993,1994) were combined with data from Vila (December 1993, January 1995). For details of scores see Methods.

Month	Frequency (%) of birds with score						n
	0	1	2	3	4	R	
April	27.3	18.2	54.5				11
May		4.8	9.5	21.4	64.3		42
June	7.9	2.6	4.8	14.8	69.8		189
July			1.3	3.9	23.4	71.4	77
August	20.1	2.4	2.4	5.3	20.7	49.1	169
September	10.1	8.2	19.7	25.4	36.6		366
October		2.4	7.3	19.5	70.7		41
November				5.0	77.5	17.5	40
December			1.3		46.1	52.6	76
January	41.7		4.2	8.3		45.8	24

TABLE 5.3. - Characteristics of eggs from the two seasonal populations of Band-rumped storm petrel breeding in the Azores. Values are mean±1s.e., sample size bracketed and range below (ns-not significant; ***-$p<0.001$).

Character	Hot season	Cool season	t-test
Length (mm)	32.0±0.2 (23) 29.9-33.2	33.6±0.1 (55) 31.5-35.5	6.77***
Breadth (mm)	24.2±0.1 (23) 23.7-25.3	24.4±0.1 (55) 22.7-25.7	1.37 ns
Mass (g) [a]	9.8±0.2 (20) 8.8-11.0	10.9±0.2 (22) 8.8-12.3	4.05***
Volume (cm^3) [b]	9.6±0.2 (23) 8.7-10.8	10.3±0.1 (55) 8.8-11.8	3.40***

[a] only values taken close to peak laying periods (hot season: June; cool season: October).
[b] external volume: $V=0.512*L*B^2$ (Stonehouse 1966).

5.3.1.4. Chick stage

The earliest dates on which chicks were observed on Baixo/Praia were 5 July and 19 August 1993 (n=9 chicks), and by 21 September 1993 eight had fledged, except one that was abandoned and starving to death (wing=81mm, weight=24g). Later, one chick of 51g (of ca. 34 days old) was found on 14 November. On Vila, no chicks were present on 19 October 1994, in 40 nest sites where laying was known from the 1993 cool season, while on 4 December 1993, 33 out of 47 nest sites had chicks and 14 had eggs in incubation. Hatching eggs were observed on 5 and 22 July 1993 on Praia and 4 December 1993 on Vila. Pre-fledglings and fledglings were observed only in August (Baixo and Praia) and January (Vila).

Measurements of chicks obtained on 4 visits are given in Table 5.4, together with estimated ages. The stage of development of chicks from July 1993 and from December 1993 is identical and mean wing length, mass and age did not differ significantly between the two samples (t-tests, $p>0.05$). Breeding is apparently less synchronous in the cool season, as indicated by the overall wider range of chick body size in cool season compared to the hot season. Chicks from the cool season were slightly heavier than chicks from the hot season for the same wing length (Fig. 5.1).

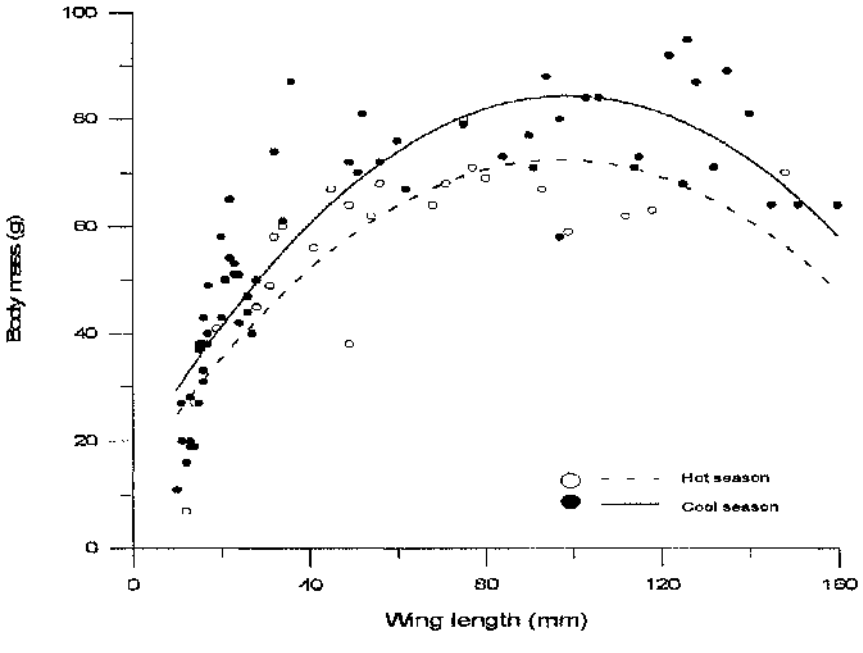
TABLE 5.4. - Mensural characters and estimated age of Band-rumped storm petrel chicks from the two seasonal populations breeding in the Azores. Values are mean±1s.e. and range below.

Population (colony)	Period	n	Wing (mm)	Mass (g)	Age[a] (days)
Hot season (Baixo/Praia)	19-22/07/93	6	20.2±3.7 12-32	33.5±7.9 7-58	21±14.9 1-41
	17-19/08/93	9	90.1±10.9 41-135	64.0±1.5 56-71	-
	3-8/08/94	11	67.9±10.7 28-148	62.8±3.6 38-80	-
Cool season (Vila)	2-4/12/93	32	20.4±1.8 10-51	41.1±3.0 11-74	24±14.5[b] 4-49
	10-17/01/95	31	95.1±7.5 16-160	71.6±2.8 31-95	-

[a] age estimated using the equation $Mass = 132.6*(1-0.964*e^{-0.0131*Age})$, fitted with growth data (error ca.±2 days) of young (range 10-60g) from the hot seasons in Galapagos (Harris 1969).

[b] n=29; three chicks over the weight range of the former equation (weight/wing: 72g/49mm; 70g/51mm; 74g/32mm) were excluded.

Fig. 5.1
Growth curves of chicks in hot and cool season populations of Band-rumped storm petrel breeding in the Azores. Equations are: hot season, MAS=13.40+1.22*WIN-0.0063*WIN**2, r=0.84, n=24; cool season, MAS=16.55+1.37*WIN-0.0070*WIN**2, r=0.88, n=63.



5.3.1.5. Breast molt

The occurrence of adult breast plumage molt is summarized per month in Table 5.5. These data reveal different patterns of breast feather molt between the two seasonal populations. More than 50% of hot season breeders initiate breast molt while in the colonies during July, i.e. shortly after hatching. In contrast, 50% of the cool season breeders are in breast molt at arrival at the breeding grounds in August, the frequency decreasing to less than 6% in September. Breast molt reappears in December but only reaches a high frequency in January, i.e. about a month after the peak of hatching.

5.3.1.6. Recaptures

Recaptures of ringed adults on Baixo and Praia were classified by season (hot season: March to July; cool season: September to November). Data from August were treated separately since brood patch data suggested both hot and cool season birds to be present in August.

Although 56 birds ringed in one season were recaptured in the same season in a subsequent year (Table 5.6), there was only one possible interchange between the hot and cool seasons. A bird ringed on Praia on 26 June 1993 (BP = 0) was recaptured in the same colony on 23 September 1993 (BP not recorded). Data from August suggest that birds from the hot season predominate in that month. Out of 35 birds ringed in August, 29 (83%) were recaptured in the hot season, while just 6 (17%) were recaptured in the cool season. Additionally, out of 14 birds recaptured in August, 11 (79%) were ringed in the hot season and 3 (21%) in the cool season.

Only one case of movement between colonies was recorded. An adult ringed on Praia on 24 September 1993 (BP = 3) was recaptured on Baixo in October 1993 (BP = 3).

5.3.2. Adult morphology

The reduced inter-change of birds between colonies and seasons allowed the recognition of five populations: Graciosa-Baixo-Hot (GBH), Graciosa-Baixo-Cool (GBC), Graciosa-Praia-Hot (GPH), Graciosa-Praia-Cool (GPC) and Santa Maria-Vila-Cool (SVC). Adults from Baixo and Praia were assigned to one of the two seasonal populations according to the following criteria: hot season, if ringed and recaptured between March and July; cool season, if ringed and recaptured

TABLE 5.5. - Frequency by month (Fa-absolute; Fr-relative) of mist-netted adult Band-rumped storm petrel with breast plumage molt on Baixo/Praia and Vila.

Month	Seasonal	Baixo/Praia			Vila		
	population	n	Fa	Fr(%)	n	Fa	Fr(%)
April	Hot	11	0	0	–		
May	Hot	41	0	0	–		
June	Hot	183	2	1.1	–		
July	Hot	78	41	52.6	–		
August	Hot	39	25	64.1	–		
	Cool	6	3	50.0	5	4	44.4
September	Cool	252	7	2.8	138	8	5.8
October	Cool	38	0	0	82	0	0
November	Cool	40	0	0	45	0	0
December	Cool	–			74	5	6.8
January	Cool	–			24	11	45.8

TABLE 5.6. - Numbers of mist-netted Band-rumped storm petrel adults ringed on Praia and Baixo in each season and numbers of retraps from one season to another, in the period 1990-1995 (hot season: March-July; cool season: late September-November).

	Birds retrapped in season				
	cool season 91	hot season 93	cool season 93	hot season 94	hot season 95
(birds handled)	(40)	(225)	(417)	(103)	(60)
Birds ringedin season:					
hot season 90-92 (58)	0	19	0	4	0
cool season 91 (40)		0	4	0	0
hot season 93 (166)			1	18	10
cool season 93 (391)				0	0
hot season 94 (83)					1

between September (the earliest visit in this month started on the 21st) and November; birds ringed in August were assigned to the hot or cool season populations if they were recaptured earlier than August or later than 21 September, respectively. For each population, all measurements except body mass did not differ significantly between mist-netted birds (unknown status) and incubating birds (t-tests, $p>0.05$) and were pooled. Incubating birds were significantly heavier than birds of unknown status caught during the incubation period, both in hot season (Baixo-Praia pooled: 48.4 ± 0.9 SE, $n=27$ versus 43.7 ± 0.4 SE, $n=161$, respectively; $t=5.10$, $p<0.0001$) and in the cool season (Vila: 54.1 ± 0.9 SE, $n=40$ versus 50.9 ± 0.4 SE, $n=194$, respectively; $t=3.39$, $p<0.001$), and Table 5.7 gives body mass of mist-netted birds.

Univariate statistics of characters for each population are given in Table 5.7. The simultaneous comparisons using ANOVA yielded significant differences among populations for all variables tested except TMI. Within the eight variables exhibiting significant differences, 38 significant (<0.05) pairwise comparisons were detected, with 37 (97%) representing differences between two dichotomous groups: hot season (GBH/GPH) and cool season (GBC/GPC/SVC) populations. Hot season birds are smaller than cool season birds in mensural characters expressing body size (NAP, CUL, NOS, GON, TAR and MAS), but contrarily have longer and more forked tails (TMA and FOR) and longer wing relative to body size.

Multivariate analyses were undertaken using seven mensural characters; TMI and FOR were excluded due to small sample sizes and NOS was excluded because of low consistency in measurements. The first two principal components (PC) extracted accounted for 61.0% of the total variance in the data set (Fig. 5.2). PC1 varies inversely with NAP, GON, CUL, TAR and MAS, representing a general body size character. PC2 varies inversely with TMA and WIN, representing flight shape. The spatial diagram of PC1 against PC2 (Fig. 5.2) suggests a separation in two dichotomous groups; the hot season populations having smaller body size and larger silhouette than the cool season populations. Differences in adult morphology among populations are highly significant, as indicated by MANOVA on PC1 and PC2 scores of individual birds (Wilks' Lambda $=0.568$, Rao's $R(8,1564)=63.81$, <0.0001). All of the 12 significant ($P<0.005$) pairwise comparisons (6 for PC1 and 6 for PC2) represented differences between the already mentioned dichotomous groups: hot season (GBH/GPH) and cool season (GBC/GPC/SVC).

The dimorphism between hot season and cool season birds indicated by ANOVA, PCA and MANOVA, was investigated further by stepwise discriminant

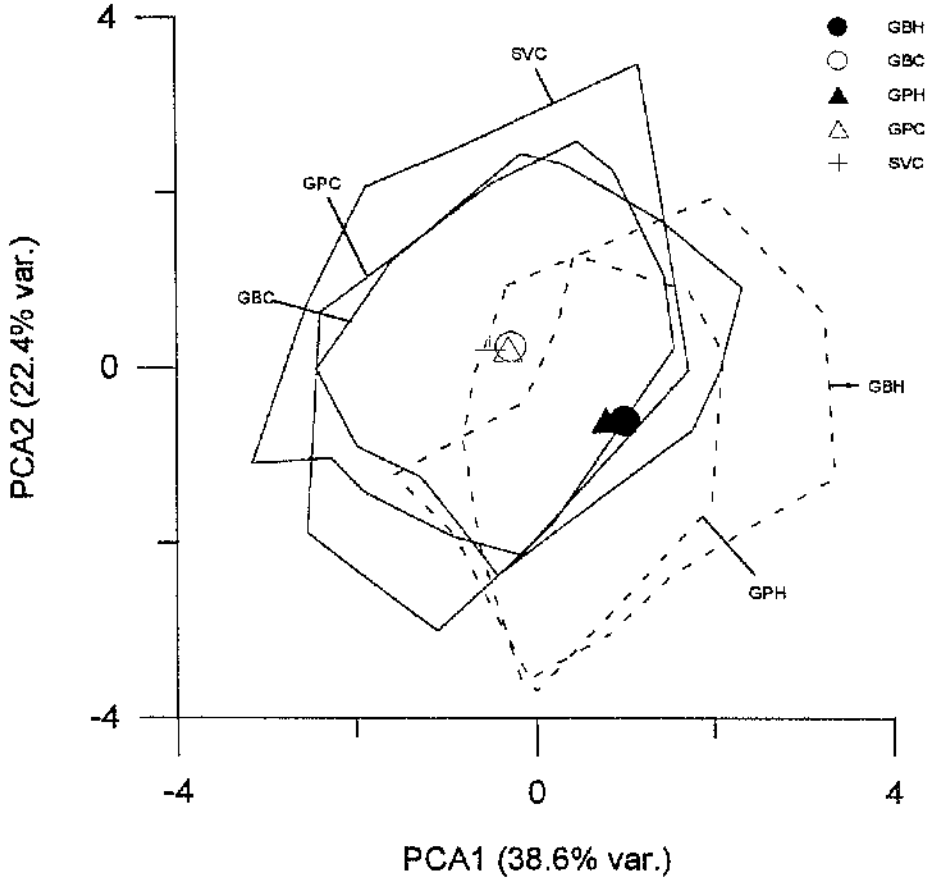
TABLE 5.7. - Means and standard errors (sample size bracketed) of characters from adult Band-rumped storm petrel of different populations breeding in the Azores. All measurements made on live birds: mist-netted and known breeders pooled, except for body mass where only data from mist-netted birds are included. For units and character acronyms see Methods and for population acronyms see Results.

	GBH	GBC	GPH	GPC	SVC	ANOVA
NAP	40.36±0.08 (113)	41.89±0.07 (156)	40.59±0.14 (40)	41.99±0.05 (239)	41.82±0.05 (250)	107.21***
CUL	14.58±0.05 (153)	14.91±0.04 (156)	14.70±0.08 (68)	14.99±0.03 (239)	15.11±0.03 (250)	28.04***
NOS	6.11±0.02 (154)	6.34±0.02 (156)	6.20±0.03 (68)	6.42±0.01 (239)	6.43±0.02 (250)	- [a]
GON	4.94±0.02 (154)	5.19±0.01 (156)	4.96±0.02 (68)	5.20±0.01 (239)	5.22±0.01 (250)	74.14***
TAR	23.54±0.06 (154)	24.06±0.06 (156)	23.54±0.10 (64)	24.03±0.04 (238)	24.18±0.04 (250)	25.11***
WIN	157.4±0.3 (154)	157.5±0.2 (191)	158.3±0.4 (72)	157.2±0.2 (240)	158.1±0.2 (298)	2.94*
TMA	75.4±0.2 (153)	73.0±0.2 (156)	75.1±0.4 (64)	73.3±0.2 (239)	72.9±0.2 (252)	29.47***
TMI	67.4±0.4 (65)	-	67.8±0.4 (63)	-	67.6±0.2 (70)	0.46[ns]
FOR	8.4±0.4 (65)	-	7.6±0.3 (63)	-	5.6±0.2 (70)	24.11***
MAS	44.0±0.4 (139)	49.2±0.4 (180)	44.0±0.5 (67)	48.5±0.2 (240)	49.3±0.3 (242)	55.33***

[a] not tested due to low consistency of measurements (see Methods)
ns - not significant, * - P<0.05, *** - P<0.0001

Fig. 5.2

Plane 1-2 of principal component analysis performed on characters of different hot and cool season populations of Band-rumped storm petrel breeding in the Azores. Diagrams are group contours and symbols centroids. For acronyms see Results, subsection adult morphology. Loadings equal or greater than 0.7 are: for PC1, Nape = -0.865, Culmen = -704, Gonys = -0.699; for PC2, Tail maximum = -0.907, Wing = -0.826.



analysis. From the seven mensural characters used (NOS, TMI and FOR excluded), WIN and TAR were removed ($F<0.20$, $p>0.50$). The result was highly significant (Canonical $R=0.698$, Wilks' Lambda $=0.513$, $\chi^2_5=523.21$, $P<0.0001$) and showed a good separation of the two groups. The discriminant function obtained correctly classified 72.8% ($n=146$) of hot season birds and 96.9% ($n=664$) of cool season birds. Although the use of unequal sample sizes is considered not to influence discriminant analysis (Tabachnick and Fidell 1989), we found that a more even distribution of correctly classified cases between populations (87.8% in hot season and 89.0% in cool season) was achieved using similar sample sizes ($n=146$ and $n=156$, respectively).

5.4. DISCUSSION

5.4.1. Breeding phenology

Comparison of data on the timing of the various events connected with breeding and recapture data, provide evidence of the existence on Baixo and Praia of two populations breeding annually, out of phase by four to five months and overlapping in colony attendance during August and early September. The periodicity of field trips did not allow precise determination of laying and hatching dates as well as incubation and fledging periods. However, these can be derived by coupling data on chick age (see Table 5.4) with mean incubation period (42 days) and mean fledging periods (70 days in hot season and 78 days in cool season) reported for the species by Harris (1969). These crude estimates of peak laying, hatching and fledging dates are, respectively: 19 May, 30 June, 8 September for the hot season population on Baixo/Praia and 1 October, 11 November, 28 January for the cool season population on Vila. Such chronology is in close agreement with the scattered field notes reported in this study. For instance, the estimated age of chicks in July and December 93 (see Table 5.4) indicated an exact 4.5 month interval between the hot breeding season population on Baixo/Praia and the cool breeding season population on Vila. Assuming that breeding is relatively well synchronized in the cool season populations on Baixo/Praia and Vila (as inferred from Table 5.1; see Results), the hot and cool season populations on Baixo/Praia seem to be also out of phase by four and half months, which is supported by the monthly distribution of brood patches (see Table 5.2). Furthermore, estimated ages of chicks in the July and December 1993 samples lie in the range of 40-45 days which is in close agreement with the spread of laying for the bulk of clutches known for the species (Harris 1969).

The breeding phenology of the Band-rumped storm petrel over its world distribution shows a perfect continuum from pure synchrony (Japan, possibly Hawaii) to strict bimodality (Galapagos, Azores, possibly Madeira), through varying levels of asynchrony (Salvages, Canary Is., Cape Verde, Ascension, St. Helena) (Mougin *et al.* 1990; this study). A possible explanation for this breeding plasticity is that populations might have distributed themselves according to an ideal free distribution in relation to four main factors: 1) availability of food resources; 2) numbers of birds, both conspecific and heterospecific, exploiting those resources; 3) availability of breeding habitat; 4) predation pressure.

The Band-rumped storm petrel shows a clear preference to breed in the cooler season, despite its pan(sub)tropical distribution. Winter breeding predominates in most places with a single population (Azores, Vila Islet: this study; Farilhões: Granadeiro pers. com.; Canary Is: Martin *et al.* 1983, Concepcion 1992; Cape Verde: Hazevoet 1995; Ascension: Allan 1962) and in all known locations with two seasonal populations (Azores, Baixo and Praia Islets: this study; Galapagos, Plaza: Harris 1969). Hence, a primary question emerges: why is the cool season widely preferred? That might arise from increased foraging efficiency comparatively with the hot season. This and other *Oceanodroma* species feed mostly on mesopelagic lanternfish (e.g. Myctophids) vertically migrating to the upper oceanic layers at night (e.g., Prince and Morgan 1987, Croxall *et al.* 1988, Warham 1990). The at-sea distribution of the species indicates a preference for areas with surface turbulence associated with particular oceanographical features, like localized upwellings (Haney 1985) and internal waves (Haney, 1987). It has been shown that consistently high winds result in drift of surface waters (down to 10-20 m) and advection of mesopelagic prey over shelf or topographic irregularities (e.g. seamounts) (Perissinotto and MacQuail 1992). Therefore, enhanced food availability for the species might be predicted in the cooler and windier season, together with a longer period of darkness to exploit their prey.

Higher inter-specific competition for food and nest sites during the hot season also may play an important role for making breeding in the cool season more advantageous for the species. Nevertheless, this seems to be unimportant at the North Atlantic colonies, although a poor knowledge of the feeding ecology and nest site selection in local seabird assemblages prevents complete testing of this hypothesis. Some circumstantial comparisons reveal that the preference for breeding in the cool season is observed even in colonies where the most direct competitors for food resources (other procellariforms specialized on mesopelagic prey like Bulwer's petrel *Bulweria bulwerii* and gadfly petrels *Pterodroma* spp.; Prince and Morgan 1987) are completely or almost absent (e.g. Farilhões; Azores, Baixo and Praia Islets). Moreover, breeding in the hot season at Madeira and especially at Salvages overlaps with the breeding of Bulwers's petrel populations estimated at several thousands of pairs (Zino and Biscoito 1994). Despite observations of competition for nest sites with other procellariforms (Bulwer's petrel, Cory's shearwater, Little shearwater *Puffinus assimilis*, White-faced storm petrel *Pelagodroma marina*) (Martin *et al.* 1984, Zino and Biscoito 1994, Hazevoet 1995, Monteiro *et al.* 1996a), this might play a relatively unimportant role.

Potential competitors for nest sites are unknown at Farilhão and the apparent overlap in nest site selection at most colonies might be minimal, as on Vila Islet (Azores; Monteiro *et al.* 1996a). Furthermore, the White-faced storm petrel, that could be a more direct competitor for nest sites and food resources, has a very distinct diet and feeding ecology (Prince and Morgan 1987) and its presence/absence does not correlate at all with the breeding seasons of the Band-rumped storm petrel at colonies where they coexist like Salvages and Cape Verde.

The apparent adaptation of Band-rumped storm petrel for the cool season should be reflected in better breeding success in this season. In Galapagos, Snow and Snow (1966) observed higher overall breeding success (hatching + fledging) in the cool season compared to the hot season, while Harris (1969) did not find differences in fledging success between seasons. However, in the more critical egg-stage, Harris (1969) found that the chances of an egg hatching decreased if left unattended and that eggs laid in the hot season were more frequently left. Higher frequency and duration of egg neglect in petrels is known to arise from poor feeding conditions (Chaurand and Weimerskirsh 1994). The subsidiarity of the hot season population might explain the lower numbers in the colonies where it coexists with the cool season population (Galapagos: Snow and Snow 1966, Harris 1969; Azores: see Methods section), in its weak representation in Ascension and Cape Verde with some out-of-season birds (Allan 1962, Hazevoet 1995) and in its complete absence in other colonies (Vila: this study; Canary Is.: Martin *et al.* 1984, Concepcion 1992). Subsidiarity suggests the prevalence of poorer conditions in the hot season and this raises the question: why do some birds breed in the apparently less favorable season at certain colonies?

The strategy might be primarily a response to reduce intra-specific competition for nest sites during the cool season in densely populated colonies, since the two seasonal populations do not differ in nest site selection and may use the same nest sites in consecutive seasons (Galapagos: Snow and Snow 1966, Harris 1969; Azores: pers. obs.), and inter-specific competition for food and nest sites seems to be relatively unimportant (see above). Intra-specific competition for nest sites is an important source of egg loss (Allan 1962, Harris 1969) and it could be the cause for displacement of birds from cool to hot season. Such a density-dependent constraint, forcing birds to share time since no further space was available for breeding, might have been strengthened recently because of an increased pressure to breed only on predator-free islets due to the historical extinction of colonies on main islands (Ascension: Ashmole *et al.* 1994; Azores:

Monteiro *et al.* 1996a). This hypothesis is supported by the smaller breeding area and apparent lower availability of good quality nest sites on Baixo and Praia colonies, where the two segregated populations occur, compared to Vila, where just the cool season population occurs. Additional evidence supporting competition for nest sites as a strong pressure in determining the breeding season of the Band-rumped storm petrel comes from Galapagos. At Isla Pitt, the congeneric Galapagos storm petrel *Oceanodroma tethys*, apparently prevents the Band-rumped storm petrel from breeding during the cool season, by occupying all the available nest sites and forcing the species to breed just in the hot season (Harris 1969).

Varying predation pressure could be another factor affecting time of breeding, allied with varying lengths of darkness, as found for Leach's storm petrel *Oceanodroma leucorhoa* in Japan (Warham, pers. com.). This appears of little importance at the Azores colonies, where the known predators are gulls on Baixo and buzzards on Vila (Monteiro *et al.* 1996a).

5.4.2. Morphological differentiation

Both univariate and multivariate analyses of adult morphology indicate a significant amount of phenotypic differentiation between the sympatric hot and cool season breeders and simultaneously a great phenotypic uniformity of allopatric breeders within the same season. Adults in the hot season are smaller and lay smaller eggs (average egg and body mass are 10% lower) and are longer-winged and longer-tailed than cool season birds. Chicks in the hot season are 15% lighter than chicks in the cool season.

The dimorphism of the two segregated Azorean populations demonstrated in this study contrasts with the monomorphism of the two segregated populations in Galapagos (Harris 1969). Local environmental influences might explain the fact that an apparently similar biological phenomenon (temporal segregation) led to different levels of phenotypic differentiation in the two archipelagos. The difference in marine climate (e.g. wind, temperature) to which the hot (subsidiary) and cool (main) season populations were subjected while breeding is far more pronounced in the Azores than in Galapagos (Harris 1969, Monteiro *et al.* 1996a). Therefore, stronger selection pressure may exist in the Azores between the two seasons. For instance, it is advantageous for birds breeding in the cool season to be larger, optimizing heat conservation and being tougher. This seasonal variation in body size agrees with Bergmann's ecogeographic rule for body size in

endotherms and correlates well with the temperatures during breeding, although it is difficult to ascertain the possible influence of the temperatures in the non-breeding grounds since they are little known (Cramp 1977). Conversely, the smaller wing and disc loading in hot season birds (the 10% reduction in body mass implies an equivalent reduction for these two variables, assuming constant wing span and area between seasons; Pennycuick 1987) might have an important adaptative significance in the hot and less windy season, since storm petrels (in contrast to most larger procellariiforms) typically forage by flying near their maximum range speed (Pennycuick 1987). So, the apparent lack of specific phenotypic adaptations for the hot season in the Galapagos might be merely a consequence of divergence in an environment with low seasonal contrasts.

The morphological differentiation observed between the two Azorean sympatric populations of the Band-rumped storm petrel is more pronounced than that between the allopatric hot season populations in the Azores and Madeira (Monteiro, unpubl. data) or than between two sympatric and seasonally segregated storm petrels classified as full species: *Oceanodroma matsudaira* (hot season) and *O. tristami* (cool season) breeding at Volcano Island (Harrison 1985, Warham 1990), and it is similar to differences between some subspecies of Leach's storm petrel in the Pacific (Power and Ainley 1986).

The strong philopatry exhibited by procellariforms reduces gene flow drastically even over short distances (e.g. Randi *et al.* 1989, Ovenden *et al.* 1991, Birt-Friesen *et al.* 1992, Austin *et al.* 1994). Nevertheless, that does not necessarily imply marked evolution since the overall potential for structural divergence among congeneric species is low (e.g. Pennycuick 1987, Warham 1990, Hazevoet 1995) resulting in subtle differences between sibling forms (e.g. Hunter 1987, Warham 1990, Bretagnolle 1995). Such constraints seem to be evident in the Band-rumped storm petrel. For instance, in the Azores there is an overall phenotypic resemblance among the allopatric cool season populations while there is significant dimorphism between sympatric ones. This suggests the existence of a strong component of environmental influence on phenotypic expression (James 1983) likely to be genetically acquired (Starck *et al.* 1995) and illustrates the danger of making inferences about the extent of allopatric speciation from comparisons of phenotypic divergence.

5.4.3. SYSTEMATICS

This study reports a case of temporal partitioning of reproduction between sympatrically dimorphic populations of a presumed monotypic (Cramp 1977) species. While there is a need for further investigation of the situation to elucidate more fully all the possible isolating mechanisms involved, from the present evidence it appears adequate to treat the seasonal populations as sibling taxa. Then, a primary question emerges: should the two populations be considered subspecies or species?

Temporal segregation of annual sympatric populations within a species is an extremely rare breeding strategy among birds and other vertebrates. However, there are a number of precedents for this situation among procellariforms at a few tropical locations: Band-rumped storm petrel (Galapagos: Harris 1969), Leach's storm petrel (Guadalupe Is.: Ainley 1980, 1983) and Dark-rumped petrel *Pterodroma phaeopygia* (San Cristobal, Galapagos: Tomkins and Milne 1991). While the Dark-rumped petrel case did not allow a confident assertion of the situation due to small sample sizes, the two cases dealing with storm petrels were handled taxonomically in different ways. Power and Ainley (1986) proposed a subspecific taxonomic arrangement for the Leach's storm petrel with the two differentiated populations of Guadalupe Is. being separate subspecies, based primarily on a canonical analysis where they formed independent clusters at the end of a well defined clinal gradient. Harris (1969) did not find significant differences in morphology between populations of Band-rumped storm petrels in Galapagos but stated that «separation of birds present in the hot and cool seasons, if associated with the young birds returning to breed at the same season of the year as they were raised, could potentially give rise to separate forms of the same species»; later three birds were found recruiting into the season they were reared (Harris 1979). Furthermore, seasonal segregation on sympatry between *Oceanodroma matsudaira* and *O. tristami* at the Bonin Islands was considered the main rationale for discriminating at the species level among the currently recognized four all-dark *Oceanodroma* of the North Pacific (Warham 1990:179).

This bimodality of reproduction observed in some tropical and subtropical populations of storm petrels might represent situations of sympatric speciation through temporal partitioning. However, this speciation mechanism requires that speciation took place in sympatry (Otte and Endler 1989, Mayr and Ashlock 1991). That apparently never has been described in birds and observing populations in sympatry today does not necessarily means that speciation took

place in sympatry (e.g. Hunter 1987). Sympatric speciation involves two types of isolating mechanisms (Mayr 1963). First, reproductive isolation, which conserves the species' characteristics, can be behavioral or anatomical in form or may involve reduced success of inter-specific breeding attempts (Mayr 1963). Secondly, ecological segregation in order to avoid competitive exclusion of one species by the other, though the prevalence and importance of such competition is presently disputed (see Roughgarden 1983, Simberloff 1983, Connell 1983). Ecological isolation in bird communities can be by habitat, feeding zone or temporal segregation of feeding activity or through differences in diet and feeding behavior (Cody 1974, Hunter 1987).

The assessment of the taxonomic status of the existing sympatric forms of Band-rumped storm petrel is difficult because of a complete lack of information on genetic drift among allopatric and sympatric populations and by the poor knowledge of the population's ecology (e.g. behavior, feeding, movements). This makes it impossible to provide evidence for evolution in sympatry and to establish if they are in the process of or have achieved sympatric speciation, and, therefore, leads the discussion on systematics to speculative grounds. Since there is a coherent ecological rationale suggesting that in the Azores the hot season population has evolved from the cool season population with time as the main isolating mechanism, the two forms of Band-rumped storm petrel breeding sympatrically on Baixo and Praia may be considered sibling species under the biological species concept (BSC; e.g., Mayr 1963, Mayr and Ashlock 1991). Dimorphism may be seen as a result of reproductive isolation maintained by ecological segregation in time (this study) and diet as indicated by mercury burdens (Monteiro *et al.* 1995, unpubl. data). However, a more conservative approach would consider that the two forms are only ecological races, regarded as possible subspecies.

A secure taxonomic approach to solve the position of the Azorean seasonal populations is precluded by the current level of knowledge on the genetics, ecology and geographic variation of all the Band-rumped storm petrel populations. We hypothesize that DNA testing may show that these morphologically different groups would be better treated as sibling taxa.

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CHAPTER 7

INTRA-SPECIFIC VARIABILITY OF MERCURY CONCENTRATIONS IN SEABIRD TISSUES AND ITS IMPLICATIONS FOR ENVIRONMENTAL MONITORING: A CASE STUDY

CONTENTS

7.1. INTRODUCTION

Widespread contamination of marine ecosystems by mercury has been demonstrated in recent years (e.g. Thompson *et al.* 1992, Mason *et al.* 1994) and the consumption of seafood is regarded as one of the most critical pathways of mercury exposure in human populations (review in WHO 1990). Mercury monitoring in the marine environment is thus a priority, and biomonitors are popular because of their capacity to integrate contamination both from abiotic (through accumulation from air, water, sediment) and from biotic (through food chain amplification) compartments of the ecosystem (e.g. Phillips 1980).

Seabird tissues have been used as monitoring units since the mid 1960s and there is a growing database of mercury levels in seabird populations from the northern hemisphere (Chapter 3, hereafter Monteiro & Furness 1995). The understanding of mercury dynamics in seabirds has improved in recent years (e.g. Furness *et al.* 1986, Braune & Gaskin 1987, Thompson & Furness 1989, Lewis & Furness 1991, Burger & Gochfeld 1992, Becker *et al.* 1993a and 1994, Stewart *et al.* 1994) and this provides a good basis for their use as monitors of spatial and temporal patterns of mercury contamination in marine and other ecosystems (reviews in Walsh 1990, Furness 1993, Monteiro & Furness 1995).

Because of conservation and ethical reasons the tissues obtainable by non-destructive sampling (i.e. feathers, blood and to a certain extent eggs) are more satisfactory for monitoring. Eggs have been widely used (e.g. Focardi *et al.* 1988, Ohlendorf *et al.* 1988). Feathers, which are both chemically and physically stable (Crewther *et al.* 1965, Appelquist *et al.* 1984) and generally accumulate higher mercury levels than other tissues (Braune & Gaskin 1987, Lewis *et al.* 1993), have gained increased popularity as monitoring units (Goede & Bruin 1984, Furness *et al.* 1986, Hahn *et al.* 1993). Body feathers present lower variation in mercury concentrations than flight feathers and they provide the most consistent and reliable indication of the total plumage burden of mercury (Furness *et al.* 1986). The use of blood has received little attention both in monitoring or toxicological studies, despite its crucial role in current models for mercury dynamics in birds (Furness 1993, Monteiro & Furness 1995).

The variability of mercury concentrations in specific seabird tissues potentially reflects the effects of several intra-specific factors like moult, age, sex, season or laying sequence (reviews in Furness 1993, Monteiro & Furness 1995). Thus, accurate monitoring programs for mercury using seabirds need a

comprehensive assessment of the intra-specific baseline 'noise' in order to identify genuine environmental variation. This chapter presents the results of a study on the natural patterns of intra-specific variability of mercury concentrations in tissues of eight seabird species from the Portuguese Atlantic. Mercury concentrations are compared in a variety of tissues serving as potential monitoring units, including eggs, blood and plumage. The effects of several methodological and biological factors on intra-tissue mercury concentrations are examined and their implications for monitoring mercury using seabirds are briefly discussed. Particular emphasis is given to the relationships between mercury concentrations in blood and plumage of both adults and chicks, as a mean of assessing adequacy of current models for mercury dynamics in birds. The results from this study provided the basis for the assessment of spatial and historical patterns of mercury contamination in this sector of the Northeast Atlantic (Chapter 11). Inter-species variation of mercury concentrations will be analysed in Sub-chapter 8.2.

7.2. MATERIALS AND METHODS

The species used here are regular breeders in the Portuguese Atlantic and belong to two distinct taxonomic groups, order Procellariiformes and family Laridae. The Procellariiformes are Bulwer's petrel *Bulweria bulwerii*, Cory's shearwater *Calonectris diomedea borealis*, Little shearwater *Puffinus assimilis baroli*, White-faced storm petrel *Pelagodroma marina hypoleuca* and two temporally segregated populations of Madeiran storm petrel *Oceanodroma castro* (hot season and cool season; Chapter 5). The Laridae are Yellow-legged gull *Larus cachinnans atlantis*, Roseate tern *Sterna dougallii* and Common tern *Sterna hirundo*.

Sampling of eggs, contour feathers of adults and old chicks, down of young chicks and blood of adults and chicks was undertaken, with few exceptions, in the Azores archipelago between April 1993 and May 1995 at three multispecific seabird colonies (Vila, Praia and Baixo Islets). The exceptions are some Yellow-legged gull samples from adults obtained from other Azorean colonies (Faial Island, Topo and Cabras Islets) and all the White-faced storm petrel samples obtained from the Salvages Islands in April and June 1993. Samples of breast feathers collected in the period 1990-1992 from adults retrapped in 1993-1994 were used for inter-year comparisons within individuals. The chicks of Procellariiformes exhibit two coats of down with the exception of White-faced storm petrel

(Warham 1990) and hereafter down 1 and down 2 refer, respectively, to primary down, at hatching, that is substituted later on by the secondary down. The chicks of the White-faced storm petrel and of the three Laridae possess only one coat hereafter designated simply as down.

Plumage and blood sampling were designed to test the effects of the following factors on mercury levels: for adults, type of contour feather, developmental stage of feather, sex, season, moult and age; for chicks, parental influence on hatchling mercury burdens, type of plumage and age/growth. Additionally, samples of blood and small growing breast feathers were collected in 1994 from Cory's shearwater adults of both sexes across the breeding season and from pre-fledging chicks to look at the relationship between blood and feather mercury concentrations; such feathers were 2 to 4 cm in total length and were experimentally induced (adults and chicks) or regrowing naturally (adults). The availability of samples still allowed testing of other effects: for eggs, component and incubation stage; for adults, feather melanin content.

Sampling, tissue preparation and storage, total mercury analysis of tissues, analytical quality control, and data analysis follow the procedures described in Chapter 4. Hereafter mercury refers to total mercury, unless otherwise stated, and all concentrations are given in microgram per gram on a fresh weight basis for plumage (ppm, fw) and on a dry weight basis (ppm, dw) for eggs and blood.

7.3. RESULTS

7.3.1. INTER-TISSUE VARIATION

A comparison of mercury concentrations in some of the most readily available tissues of seabirds from the study area is shown in Table 7.1. The tissues considered are homogenised egg contents (i.e. yolk and albumen), whole blood and breast feathers from adults, and several types of plumage from chicks of varied ages (one or two types of down and breast/flank feathers).

Several patterns may be drawn from results in Table 7.1 using intra-specific ratios of mean mercury concentrations for inter-tissue comparisons (see Table 7.2). Adult breast feathers show higher mercury concentrations than blood with an average ratio of 2.2 (S.D. = 0.2, n = 4) for most species; however, this ratio rises to about 4 (4.0, 4.3) in two cases and this might arise from important

TABLE 7.1. - Comparison of mercury concentrations (μg/g) in some readily available tissues of seabirds from the study area. For explanations of plumage types see text.

Species	Growth stage	Tissue type	Mean	S.D.	n	CV(%)
Bulwer's petrel		egg content	7.1	1.3	12	18
	adults	blood	11.4	1.9	25	17
	hatchlings	breast feather	22.3	4.1	91	18
	older nestlings	down1	21.0	4.3	20	20
		down2	13.1	3.7	8	28
		breast feather	3.7	0.9	19	24
Cory's		egg content	1.9	0.4	23	22
shearwater	adults	blood	2.3	0.9	152	39
	hatchlings	breast feather	6.4	1.7	186	31
	older nestlings	down1	5.0	1.0	61	20
		down2	2.5	0.4	27	16
		breast feather	0.8	0.4	62	50
Little		egg content	1.7	0.9	14	52
shearwater	adults	blood	1.4	1.1	21	79
	hatchlings	breast feather	3.1	1.0	82	32
	older nestlings	down1	6.9	2.0	8	29
		down2	2.2	0.8	15	36
		breast feather	0.6	0.4	5	67
White-faced	adults	breast feather	3.0	1.1	35	37
storm petrel	pre-fledglings	breast feather	1.5	0.4	27	27
hot season		egg content	3.6	0.7	6	18
Madeiran storm	adults	blood	5.4	0.7	8	13
petrel	hatchlings	breast feather	11.1	3.3	100	30
	older nestlings	down1	12.2	3.2	9	26
		down2	6.5	2.1	11	32
		breast feather	2.4	0.7	19	29
cool season		egg content	5.1	1.2	23	29
Madeiran storm	adults	blood	4.3	1.2	22	28
petrel	hatchlings	breast feather	17.4	4.7	130	27
	older nestlings	down1	8.8	3.2	18	36
		down2	4.2	1.2	27	29
		breast feather	2.3	0.7	28	30
Yellow-legged		egg content	1.1	0.6	22	50
gull	adults	blood	1.1	0.9	9	82
	hatchlings	breast feather	4.7	1.7	44	36
	older nestlings	down	2.1	0.8	36	38
		breast feather	2.3	1.0	35	43
Roseate tern		egg content	1.6	0.4	16	26
	adults	breast feather	2.0	0.7	22	35
	hatchlings	down	2.9	0.7	26	24
	older nestlings	breast feather	1.4	0.5	72	36
Common tern		egg content	1.9	0.5	16	26
	adults	breast feather	2.1	0.6	27	29
	hatchlings	down	3.6	1.4	38	39
	older nestlings	breast feather	1.5	0.5	72	33

TABLE 7.2. - Inter-tissue ratios for some mean mercury concentrations given in Table 7.1.

Species	adult breast : blood	adult blood at laying : egg	adult breast : hatchling down	adult breast : chick breast	hatchling down : egg	hatchling down : chick breast
Bulwer's petrel	2.0	1.5	1.1	6.0	3.0	5.7
Cory's shearwater	2.4	1.4	1.1	6.3	2.6	6.3
Little shearwater	2.2	1.4	0.5	5.2	4.1	11.5
White-faced storm petrel	–	–	–	2.0	–	–
hot season Mad. storm petrel	2.1	1.6	0.9	4.6	3.4	5.1
cool season Mad. storm petrel	4.0	1.8	2.0	7.6	1.7	3.8
Yellow-legged gull	4.3	–	2.2	2.0	1.9	0.9
Roseate tern	–	–	0.7	1.4	1.8	2.1
Common tern	–	–	0.6	1.4	1.9	2.4

seasonal influences on blood mercury concentrations (see below). The ratio adult blood at laying (using blood concentrations given below in Tables 7.8 and 7.9) and egg concentrations shows little variation and averages 1.5 (S.D.=0.2, n=5) for the Procellariiformes. Concentrations in down of hatchlings are higher than in the egg by an average ratio of 3.0 (S.D.=0.9, n=5) for the Procellariiformes and an average ratio of 1.9 (S.D.=0.1, n=3) for the Laridae. The ratios of concentrations in adult breast feathers and down of hatchlings show marked variation (range=0.5-2.2) even within taxonomic groups. Concentrations in adult breast feathers are markedly higher than in nestling breast feathers for species with two coats of down (average ratio: 5.9, S.D.=1.1, n=5) but just slightly higher for species with just one coat of down (average ratio: 1.7, S.D.=0.3, n=4). The chicks of Procellariiformes with two coats of down show concentrations in the first coat about twice those in the second coat (average ratio: 2.1, S.D.=0.6, n=5). Again for Procellariiformes chicks, mercury concentrations in the first or single coat are over five times higher than in nestling breast feathers (average ratio: 5.2, S.D.=1.1, n=4; Little shearwater excluded) while they are about the same to twice in the Laridae, which have just one coat (ratio range: 0.9-2.3).

Mercury concentrations show a noteworthy variability within each tissue type, with most coefficients of variation ranging between 20 and 40% (see Table 7.1) and apparently without any clear relation to species or type of tissue. Some factors potentially accounting for this are analysed in the following sections.

7.3.2. Eggs

7.3.2.1. Component

Mercury is unequally distributed among egg components (Table 7.3). Concentrations are much higher in the albumen than in the yolk and were below detection limits in the shell. The mercury burden in the albumen represented over 80% of the egg content burden in all species considered (Cory's shearwater: 88.9% $\pm$3.2 S.D., n=5).

7.3.2.2. Incubation stage and condition

Mercury concentrations in whole egg content did not differ significantly at different stages of incubation, i.e. between fresh eggs (with yolk and albumen)

TABLE 7.3. - Comparison of mercury concentrations (µg/g dw; mean ±S.D.) in egg components and whole egg contents in two seabird species.

Species	n	Yolk	Albumen	Shell	Egg content*
Bulwer's petrel	1	1.3	26.4	<0.005**	7.9
Cory's shearwater	5	0.3 ±0.07	5.7 ±0.8	<0.005	1.9 ±0.2
Yellow-legged gull	2	0.3, 0.5	2.5, 5.1	<0.005	1.0, 1.8
Common tern	2	0.06, 0.3	1.7, 2.2	<0.005	0.8, 0.9

* Concentrations of egg content were calculated as a sum of the product of concentrations and weights of yolk and albumen.

** Detection limit.

TABLE 7.4. - Comparison of mercury concentrations (µg/g dw; mean ±S.D., sample size bracketed) between eggs with different incubation stages for several species.

Species	Fresh	Pre-hatching	t-test
Cory's shearwater	1.9 ±0.4 (23)	2.2 ±0.3 (4)	$t_{25}=1.30$, P=0.21
Yellow-legged gull	1.1 ±0.6 (15)	1.2 ±0.5 (7)	$t_{20}=0.60$, P=0.55
Roseate tern	1.6 ±0.4 (10)	1.6 ±0.4 (6)	$t_{14}=0.13$, P=0.90

and pre-hatching eggs (with feathered embryo) for all the species tested (Table 7.4). Rotten eggs may produce inconsistent results, as indicated by low mercury concentrations determined in single rotten eggs of Bulwer's petrel (1.4 µg/g dw) and Roseate tern (0.3 µg/g dw) but comparable to fresh eggs for a single rotten Cory's shearwater egg (1.8 µg/g dw).

7.3.3. Adults

7.3.3.1. Type of contour feather

Mercury concentrations in different types of contour feathers (breast, back, scapulars, flank/side, axillaries) from individual adults sampled in the same month were compared by randomised block ANOVA (i.e. 2-way ANOVA with individual

as the random factor; Zar 1984) or paired t-test for comparison of two feather types (Table 7.5). Differences were significant among feather types for Little shearwater and the two subspecies of Madeiran storm petrel, and approached significance for Cory's shearwater.

Additional pairs of breast and back feather samples confirm the general tendency for slightly higher mercury levels in breast feathers among Procellariiformes that emerges from Table 7.5, with significant differences between means (breast vs. back) for Bulwer's petrel (22.9 $\pm$4.4 S.D. vs. 19.3 $\pm$4.6 S.D. µg/g dw, $t_{20}=3.99$, $P<0.001$) and Cory's shearwater (4.3 $\pm$1.6 S.D. vs. 3.5 $\pm$1.5 S.D. µg/g dw, $t_{20}=3.63$, $P<0.005$).

Breast (white) and back (dark brown) feathers growing simultaneously in adult Cory's shearwaters show similar mean mercury concentrations (breast: 5.0 µg/g fw $\pm$1.4 S.D.; back, 5.1 µg/g fw $\pm$1.4 S.D.; paired $t_8=0.96$, $P=0.34$). This suggests that the variation of mercury levels among different types of contour feathers does not relate to their position on the body or to their melanin content, but to differences in timing of moult.

7.3.3.2. Fully- and partly-grown feathers

Mercury concentrations in pairs of fully- and partly-grown breast feathers collected from individual adults on breast feather moult (for Procellariiformes this overlaps with the breeding season to a great extent; Sub-chapter 2.2) differ significantly in two out of four species tested (Table 7.6). The mercury ratios fully:partly grown are identical in Bulwer's petrel and hot season Madeiran storm petrel shortly after the start of breast feather moult in July (1.7 and 1.5, respectively). The same ratio approaches 1 for Cory's shearwater sampled in September, i.e. over three month after the start of breast feather moult, and for cool season Madeiran storm petrel shortly after the start of such moult.

7.3.3.3. Sex

The effect of sex on tissue mercury levels was tested for breast feather and blood samples of Cory's shearwater. Males show significantly higher concentrations than females both in breast feathers (5.9 $\pm$1.7 S.D. vs. 4.9 $\pm$1.6 µg/g dw, respectively; $t_{184}=4.20$, $P<0.0001$) and blood (2.4 $\pm$1.0 vs. 2.1 $\pm$0.8 S.D. µg/g dw, respectively; $t_{150}=1.99$, $P<0.05$).

TABLE 7.5. - Comparison of mercury concentrations (µg/g fw; mean ±S.D.) in different types of contour feathers in adults from several species.

Type of contour feather	Bulwer's petrel	Cory's shearwater	Little shearwater	hot season Mad. storm petrel	cool season Mad. storm petrel	Yellow-legged gull
	June (n=10)	March (n=15)	April (n=15)	June (n=10)	September (n=10)	March (n=15)
Breast	22.1 ±4.7	3.8 ±1.2	3.3 ±1.1	13.1 ±2.3	19.6 ±5.0	4.0 ±1.6
Back	19.4 ±5.6	3.1 ±1.4	2.8 ±0.8	11.5 ±3.6	12.4 ±2.7	4.2 ±0.8
Scapular	19.4 ±6.7	3.3 ±1.6		12.4 ±4.3	17.5 ±6.2	
Flank	19.7 ±5.4	3.2 ±1.1		12.2 ±3.6	21.1 ±4.8	
Axillaries	19.4 ±3.9	3.4 ±1.4		9.5 ±2.0	10.9 ±1.5	
ANOVA:	$F_{4,36}=1.04$ P=0.40	$F_{4,56}=2.23$ P=0.077	$t_{14}=2.30$ P<0.05	$F_{4,36}=3.05$ P<0.05	$F_{4,36}=10.3$ P<0.0001	$t_{14}=1.17$ P=0.69

TABLE 7.6. - Comparison of mercury concentrations (µg/g dw; mean ±S.D.) in pairs of fully- and partly grown breast feathers from individual adults of several species.

Species	Month	n	Fully-grown	Partly-grown	paired t-test
Bulwer's petrel	Jul-Aug	8	21.9 ±3.9	36.8 ±7.2	$t_7=7.89$ P<0.0001
Cory's shearwater	Sep	9	6.6 ±1.9	6.5 ±1.5	$t_8=0.20$ P=0.85
hot season Madeiran storm petrel	Jul-Aug	23	11.8 ±4.6	18.0 ±6.1	$t_{22}=3.35$ P<0.005
cool season Madeiran storm petrel	Jan	21	18.9 ±4.8	21.5 ±6.0	$t_{20}=1.56$ P=0.13

7.3.3.4. Season

Mercury concentrations in breast feather samples collected across the breeding season for a variety of species showed significant variations in three out of eight cases tested (Table 7.7) and the same was observed with mercury concentrations in blood samples in two out of four cases tested (Table 7.8). A comparison of mercury concentrations in breast feathers and blood of male and female Cory's

TABLE 7.7. - Comparison of mercury concentrations (µg/g fw; mean ±S.D., sample size bracketed) in breast feathers of adults from several species across the breeding season.

Month	Bulwer's petrel	Little shearwater	White-faced storm petrel	hot season Mad. storm petrel	cool season Mad. storm petrel	Yellow-legged gull	Roseate tern	Common tern
Jan		2.5 ±0.4 (12)			17.9 ±4.7 (19)			
Feb						3.1 ±0.8 (4)		
Mar		3.2 ±1.3 (12)	3.0 ±0.8 (13)			3.5 ±2.0 (14)		
Apr		2.9 ±1.0 (12)		8.6 ±1.6 (10)		6.2 ±1.3 (5)		
May	23.6 ±3.5 (18)	2.9 ±0.6 (12)		10.8 ±2.2 (15)		5.6 ±0.8 (8)		
Jun	22.2 ±5.0 (13)		2.9 ±0.9 (20)	11.4 ±2.8 (39)			2.0 ±0.8 (14)	2.2 ±0.6 (7)
Jul	22.3 ±3.4 (21)			9.8 ±3.2 (18)		4.5 ±0.9 (4)	2.0 ±0.5 (8)	2.1 ±0.6 (19)
Aug	22.8 ±4.9 (16)	3.6 ±1.4 (10)		13.1 ±4.7 (18)	20.9 ±3.6 (15)	5.4 ±0.6 (9)		
Sep		3.6 ±0.7 (13)			17.7 ±4.7 (38)			
Oct		3.4 ±0.6 (8)			16.5 ±4.8 (15)			
Nov					14.3 ±3.5 (27)			
Dec					18.7 ±4.4 (16)			
ANOVA	$F_{4,64}=0.33$ $P=0.86$	$F_{6,72}=2.37$ $P<0.05$	$t_{31}=0.37$ $P=0.99$	$F_{4,95}=4.30$ $P<0.005$	$F_{4,84}=0.44$ $P=0.78$	$F_{5,38}=5.68$ $P<0.001$	$t_{20}=0.016$ $P=0.99$	$t_{24}=0.26$ $P=0.78$

TABLE 7.8. - Comparison of mercury concentrations (µg/g dw; mean ±S.D., sample size bracketed) in blood of adults from several species across the breeding season.

Month	Bulwer's petrel	Little shearwater	hot season Mad. storm petrel	cool season Mad. storm petrel
May	12.7 ±1.6 (4)	2.4 ±0.8 (9)		
Jun	11.0 ±1.0 (6)		5.7 ±0.6 (3)	
Jul	10.6 ±2.1 (7)			
Aug	11.6 ±2.3 (8)		5.3 ±0.8 (5)	
Sep		0.5 ±0.1 (11)		3.5 ±0.7 (11)
Oct				5.2 ±1.0 (11)
	$F_{4,21}=0.83$[a] P=0.52	Z=3.76[b] P<0.0005	$t_8=0.84$[c] P=0.43	Z=3.51[b] P<0.0005

[a] ANOVA, [b] Mann-Whitney, [c] t-test.

shearwaters across the breeding season also showed highly significant effects of month of sampling and sex (Table 7.9, Fig. 7.1). Overall, the significant seasonal differences in breast feather mercury concentrations correspond to a lowest concentration averaging 68% of the highest concentration, except for the Yellow-legged gull where this percentage is 50%. The seasonal fluctuations of mercury concentrations in blood samples are more pronounced than those observed in breast feathers, with the lowest concentrations averaging 30 and 21% of the highest concentrations in Cory's and Little shearwater, respectively.

Mercury concentrations in growing breast feathers of Cory's shearwater (see methods section) collected monthly from May to October decrease in a highly significant way across months and significantly between sexes as indicated by 2-way ANOVA (Table 7.9, Fig. 7.2). Such monthly decline of mercury concentrations in growing breast feathers (BR) shows a noteworthy overall resemblance to the monthly decline of blood (BL) mercury concentrations (see Fig. 7.1B) and both are best described by a power model (pooled sexes, May-October) with almost identical decay constants:

$$BR = 136.59 * MONTH^{-1.474}, r=0.83, n=103 \quad (7.1)$$

$$BL = 36.31 * MONTH^{-1.458}, r=0.75, n=129 \quad (7.2)$$

TABLE 7.9. - Comparison of mercury concentrations (mean ±S.D., sample size bracketed) in breast feathers and blood of male and female Cory's shearwaters across the breeding season.

Month	Breast feathers (µg/g fw)		Blood (µg/g dw)		Growing breast feathers (µg/g fw)	
	Male	Female	Male	Female	Male	Female
Mar	5.9 ±1.9 (21)	4.9 ±1.7 (22)	2.6 ±0.4 (12)	2.3 ±0.7 (11)		
Apr	6.1 ±0.9 (6)	4.7 ±1.3 (5)				
May	6.1 ±1.5 (17)	5.0 ±1.4 (9)	3.5 ±0.9 (11)	3.3 ±0.5 (10)	12.7 ±2.7 (10)	10.8 ±1.2 (6)
Jun	5.4 ±1.7 (10)	5.1 ±1.4 (9)	2.9 ±0.6 (13)	2.4 ±0.3 (22)	10.3 ±1.9 (8)	11.1 ±0.9 (8)
Jul	4.8 ±2.0 (16)	4.1 ±1.3 (15)	3.0 ±0.3 (11)	2.5 ±0.4 (11)	9.5 ±1.9 (11)	7.8 ±1.3 (11)
Aug	6.3 ±1.5 (19)	5.1 ±1.7 (18)	2.0 ±0.9 (11)	1.2 ±0.3 (9)	6.3 ±1.9 (10)	5.1 ±1.1 (8)
Sep	7.0 ±1.3 (14)	5.6 ±1.6 (15)	1.3 ±0.4 (10)	0.9 ±0.3 (10)	5.0 ±1.1 (10)	4.3 ±0.9 (10)
Oct			1.2 ±0.5 (8)	1.7 ±0.3 (3)	4.4 ±1.8 (8)	4.8 ±0.6 (3)
2-way ANOVA, effects:						
Month	$F_{6,182}=3.65$, $P<0.005$		$F_{6,138}=47.21$, $P<0.0001$		$F_{5,91}=56.98$, $P<0.0001$	
Sex	$F_{1,182}=16.37$, $P<0.0001$		$F_{1,138}=11.33$, $P<0.0001$		$F_{1,91}=4.39$, $P<0.05$	

Fig. 7.1
Mercury concentrations in breast feathers (A) and blood (B) of male and female Cory's shearwaters across the breeding season (mean ±1 S.D.).

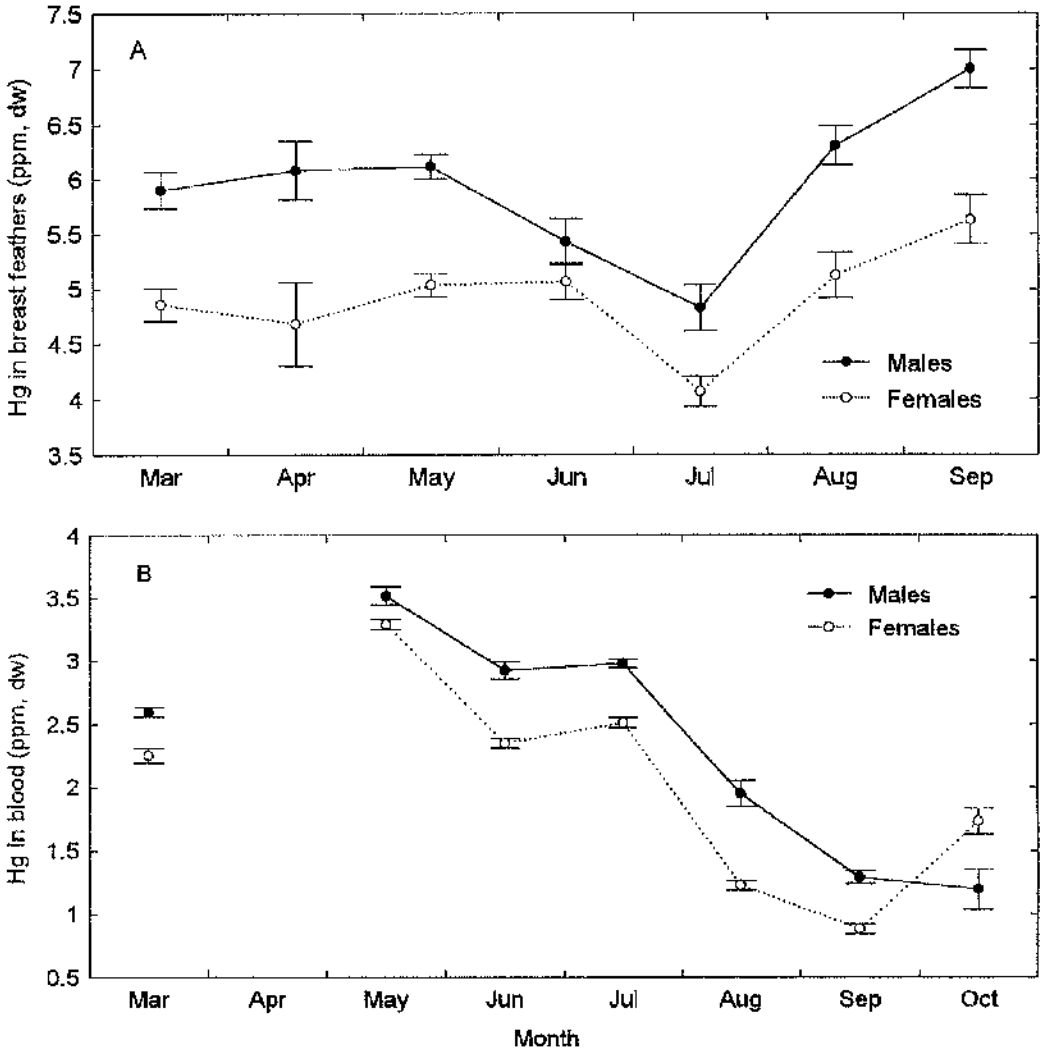


Fig. 7.2
Mercury concentrations in growing breast feathers of male and female Cory's shearwaters across the breeding season (mean +1 S.D.).

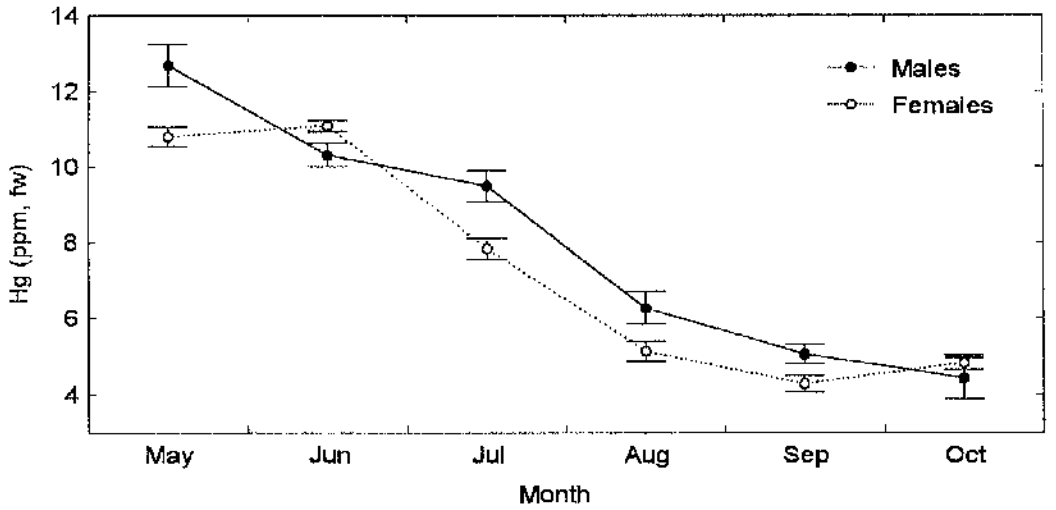
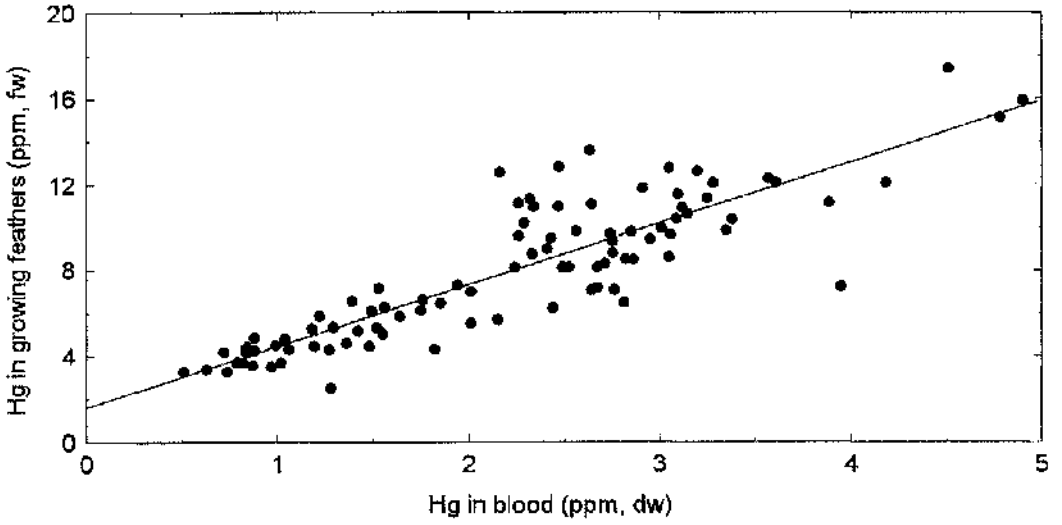


Fig. 7.3
Relationship between mercury concentrations in growing breast feathers (BR) and blood (BL) collected from adult Cory's shearwaters from May to October.



The monthly ratio of mean mercury concentrations in growing feathers and blood shows little variation and averages 3.7 (S.D. = 0.5, range 3.1-4.3, n = 6).

7.3.3.5. Blood-feather relationship

The relationship between mercury concentrations in growing breast feathers (BR) and blood (BL) collected simultaneously from the same individual Cory's shearwaters from May to October is highly significant ($r = 0.98$, $F_{1,102} = 2432.3$, $P < 0.0001$) and best described by a linear equation (Fig. 7.3):

$$BR = 3.47 \pm 0.07 S.E. * BL \qquad (7.3)$$

7.3.3.6. Moult

Mercury concentrations in blood (BL) of adult Cory's shearwaters (pooled sexes, due to small sample sizes) were found to decrease significantly with the progress of primary moult (SC) at a similar rate both in September ($r = 0.55$, $F_{1,18} = 8.00$, $P < 0.05$) and October ($r = 0.79$, $F_{1,9} = 14.65$, $P < 0.005$) (Fig. 7.4):

Sep: $Hg = 1.25 \pm 0.09 S.E. - 0.053 \pm 0.019 S.E. * SC$ (7.4)

Oct: $Hg = 1.95 \pm 0.19 S.E. - 0.053 \pm 0.014 S.E. * SC$ (7.5)

Although the two lines have identical slopes they differ significantly (ANCOVA, $F_{1,28} = 20.95$, $P < 0.0001$) due to different elevations.

Fig. 7.4

Relationship between mercury concentrations in blood (Hg) and primary moult score (SC) for adult Cory's shearwaters in September and October.

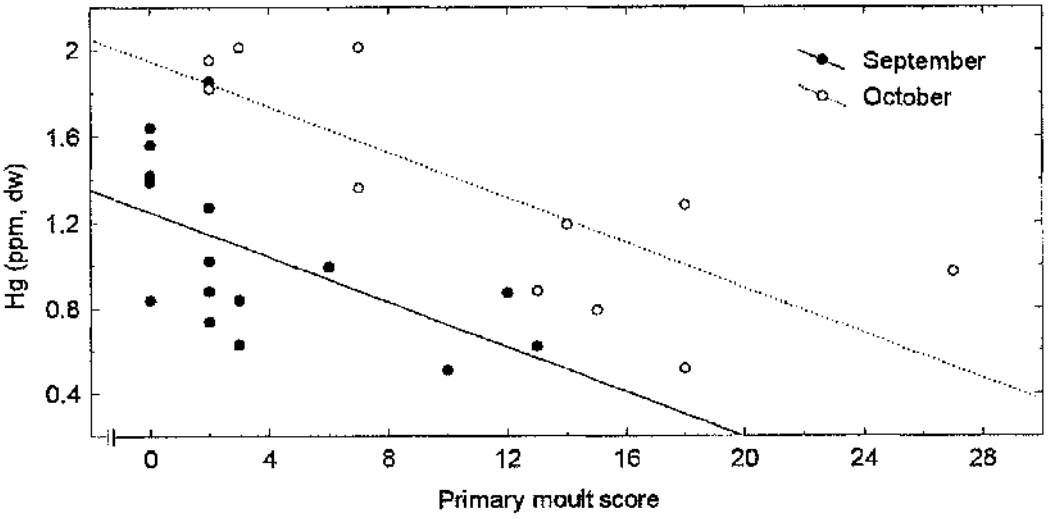


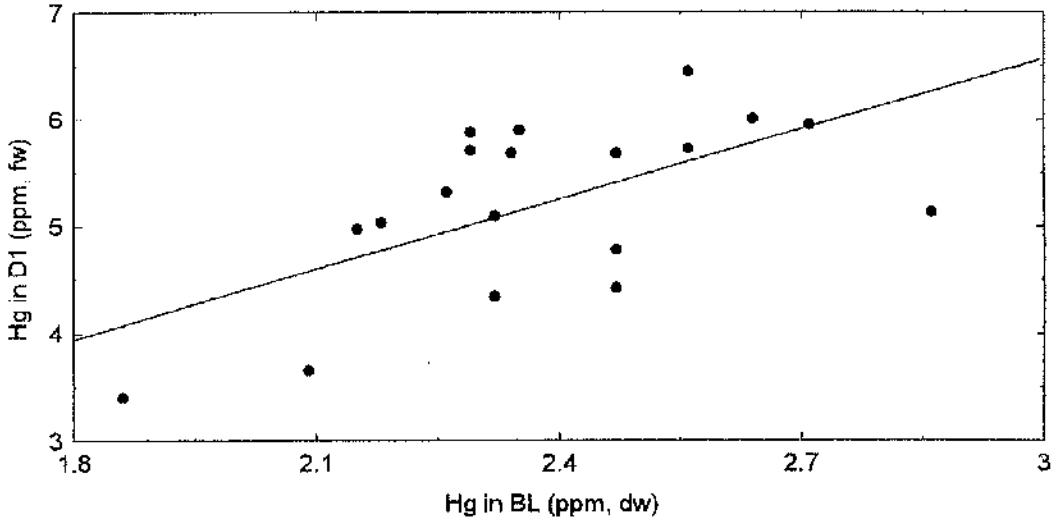
TABLE 7.10. - Comparison of mercury concentrations (µg/g dw; mean ±S.D.) in samples of breast feathers from the same individuals collected in $year_n$ and in $year_{n+(1-3)}$.

Species	n	$Year_n$	$Year_{n+(1-3)}$	paired t-test	correlation
Bulwer's petrel	20	22.1 ±3.4	22.1 ±4.1	$t_{19}=0.0026$ $P=0.998$	$r=0.33$ $P=0.15$
Cory's shearwater	36	5.2 ±1.9	4.9 ±1.2	$t_{36}=0.70$ $P=0.49$	$r=0.27$ $P=0.14$
hot season Mad. storm petrel	24	9.1 ±2.0	10.1 ±2.5	$t_{23}=1.80$ $P=0.086$	$r=0.34$ $P=0.10$
cool season Mad. storm petrel	19	17.5 ±4.7	17.5 ±7.3	$t_{18}=0.010$ $P=0.992$	$r=0.48$ $P<0.05$

7.3.3.7. Individual variation between seasons

Mean mercury concentrations in breast feather samples obtained at the same month (to control for season effects) in year *n* (1990-1993) and in one subsequent year *n plus 1 to 3* (1992-1994) from the same individual adults did not vary significantly for all species tested (Table 7.10). Pearson product-moment correlations of concentrations in the two years were significant for cool season Madeiran storm petrel and approached significance for the remaining species (Table 7.10).

Fig. 7.5
Relationship between mercury concentrations in down1 of hatchlings (D1) and blood (BL) of parent females near laying for Cory's shearwater.



7.3.4. CHICKS

7.3.4.1. Parental influence on mercury concentration in hatchlings

The influence of parental mercury burden on hatchling mercury burden (via the egg) was assessed for Cory's shearwater, using down1 of hatchlings (D1) less than two weeks old and blood of the parental female (BL) collected within a week after laying of the single egg. The two variables show a significant positive linear relationship ($r = 0.99$, $F_{1,18} = 1217.5$, $P < 0.0001$; Fig. 7.5):

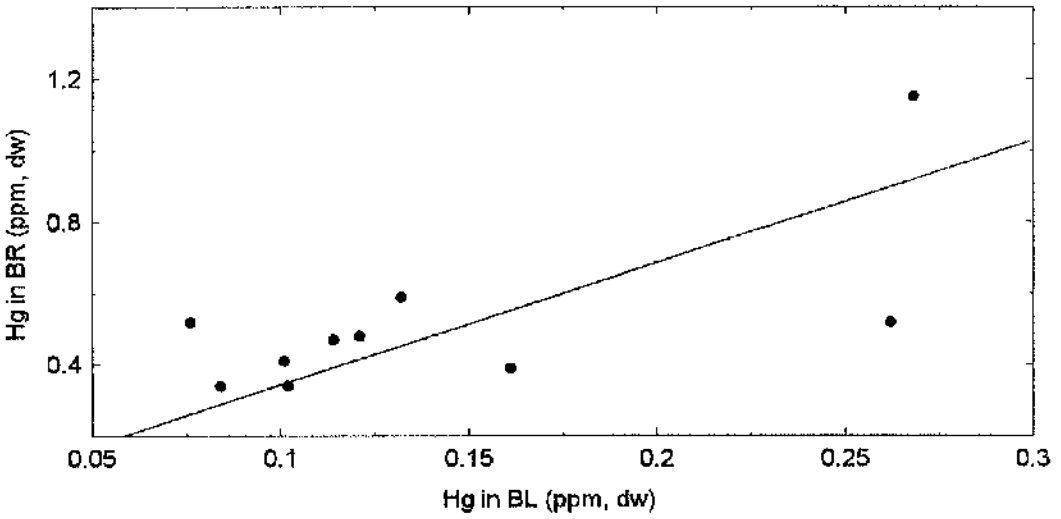
$$D1 = 2.19 \pm 0.03 S.E. * BL \quad (7.6)$$

7.3.4.2. Type of plumage

Mercury concentrations in different plumage types of Procellariiformes chicks (i.e. down 1, down2, breast/flank; see Table 7.1) differ in a highly significantly way as indicated by Kruskal-Wallis tests for all cases tested: Bulwer's petrel ($H_{2,n=47} = 36.74$, $P < 0.0001$), Cory's shearwater ($H_{2,n=150} = 127.28$, $P < 0.0001$), Little shearwater ($H_{2,n=28} = 21.64$, $P < 0.0001$), hot season ($H_{2,n=48} = 38.79$, $P < 0.0001$) and cool season ($H_{2,n=73} = 53.38$, $P < 0.0001$) Madeiran storm petrel.

Mercury concentrations in down and breast/flank feathers of Laridae chicks (see Table 7.1) did not differ significantly for Yellow-legged gull (Mann-Whitney test, $Z_{65} = 1.48$, $P = 0.14$) but were highly significantly different for Roseate tern

Fig. 7.6
Relationship between mercury concentrations in growing breast feathers (BR) and blood (BL) collected simultaneously from chicks of Cory's shearwater.



(M-W test, $Z_{88}=6.90$, $P<0.0001$) and Common tern (M-W test, $Z_{93}=8.06$, $P<0.0001$).

7.3.4.3. Blood-feather relationship

Mercury concentrations in developing plumage (growing breast feathers) of chicks (BR) and blood (BL) collected simultaneously from the same individuals are significantly correlated ($r=0.95$, $F_{1,9}=79.65$, $P<0.0001$) and the relationship is best described by the linear equation (Fig. 7.6):

$$BR = 3.43 \pm 0.38 \text{ S.E.} * BL \qquad (7.7)$$

7.3.4.4. Age and growth

The patterns of differences in mercury concentrations among plumage types of chicks with different developmental stages, mentioned above, are illustrated in figures 7.7 and 7.8. This led to an assessment of the influence of nestling's age (expressed as wing length) on plumage mercury burdens by performing regression analysis between mercury concentrations in down or in breast/flank feathers (isolated or pooled) against nestling wing length.

Fig. 7.7
Variation of mercury concentrations in different plumage types with age (expressed as wing length) of Cory's shearwater chicks.

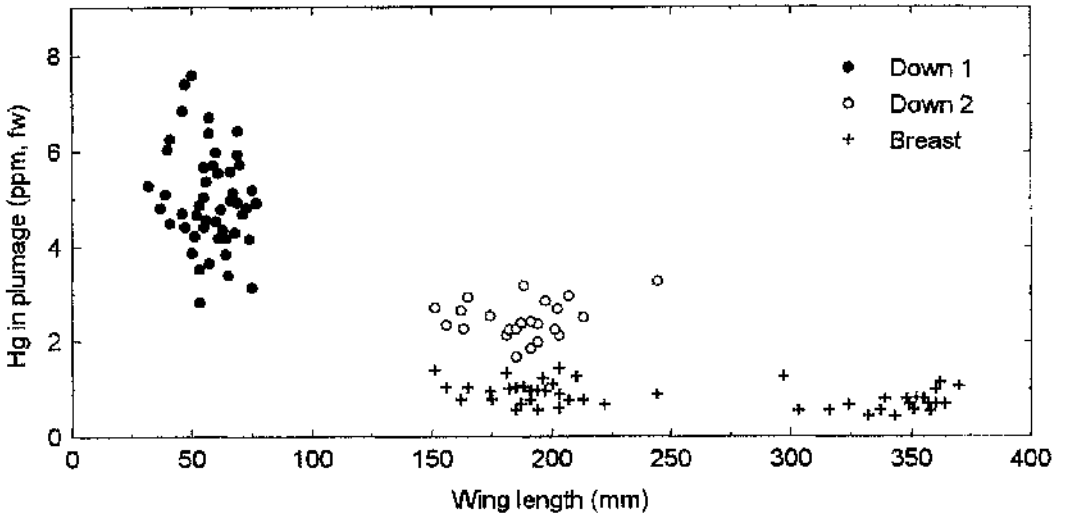
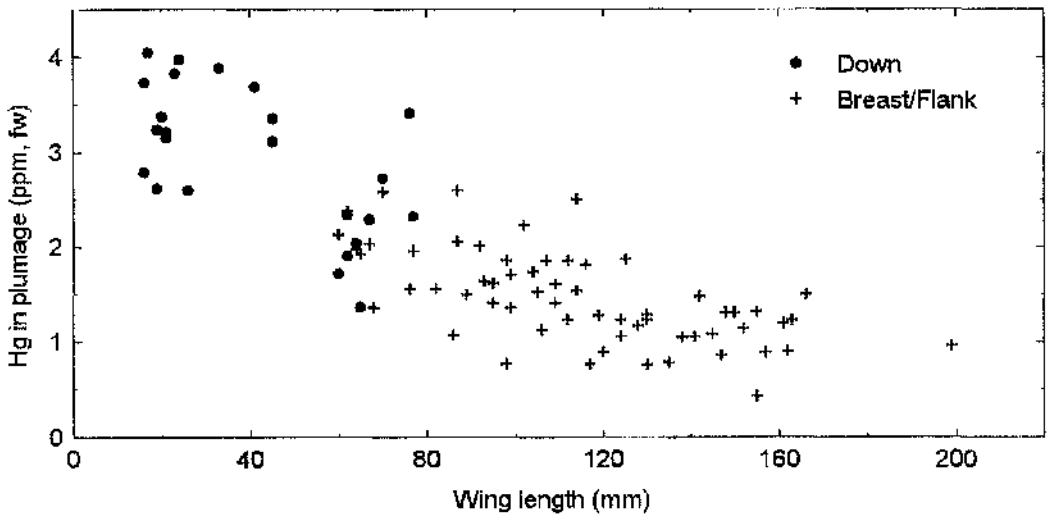


Fig. 7.8
Variation of mercury concentrations in different plumage types with age (expressed as wing length) of Roseate tern chicks.



The equations producing the best fits are given in Table 7.11 for Procellariiformes and Table 7.12 for Laridae. There is a general pattern of decreasing mercury concentrations with increasing nestling wing length. This is particularly evident with pooled down data of Procellariiformes and with pooled down+breast/flank data of terns, that show an overall remarkable similarity among the exponents of power models (i.e. decay constants) averaging 0.503 $\pm$0.058 S.D. (range 0.467-0.619, n=6; Bulwer's petrel excluded). The Yellow-

TABLE 7.11. - Models and parameters for the regressions between mercury concentrations (Hg) in plumage and age expressed as wing length (WL) for chicks of Procellariiformes.

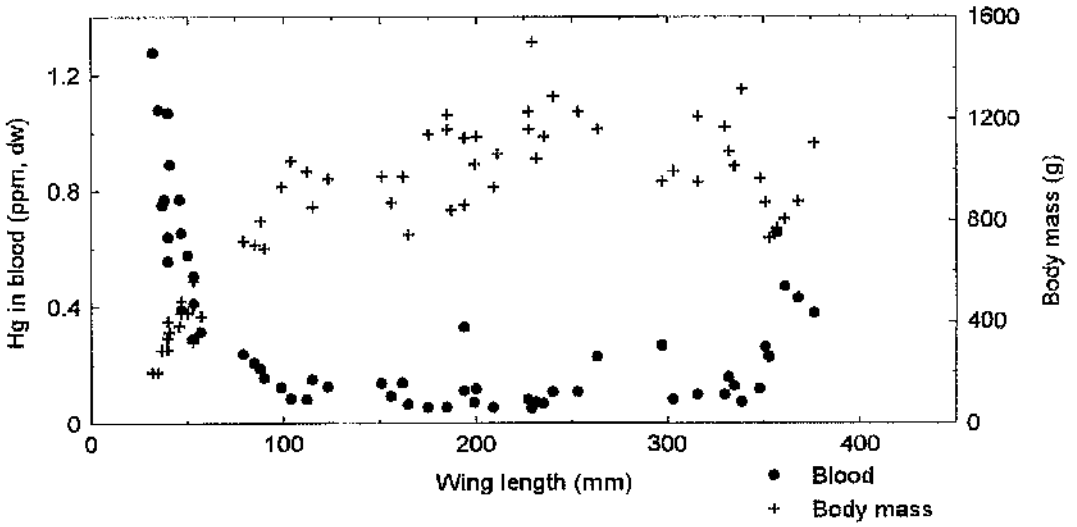
Plumage type	Species	Model	Regression parameters
Down 1	Bulwer's petrel	$Hg = a + b*WL$	$r = 0.0067$, $F_{1,17} = 0.00076$, $P = 0.98$
	Cory's shearwater	$Hg = a + b*WL$	$r = 0.038$, $F_{1,55} = 0.079$, $P = 0.78$
	Little shearwater		$r = 0.59$, $F_{1,3} = 1.60$, $P = 0.30$
	hot season Mad. storm petrel		$r = 0.71$, $F_{1,8} = 6.00$, $P = 0.050$
	cool season Mad. storm petrel		$r = 0.55$, $F_{1,15} = 6.50$, $P < 0.05$ $a = 12.1 \pm 1.5$ S.E., $b = -0.15 \pm 0.060$ S.E.,
Down 2	Bulwer's petrel	$Hg = a + b*WL$	$r = 0.25$, $F_{1,6} = 0.39$, $P = 0.56$
	Cory's shearwater		$r = 0.21$, $F_{1,25} = 1.17$, $P = 0.29$
	Little shearwater		$r = 0.51$, $F_{1,9} = 3.15$, $P = 0.11$
	hot season Mad. storm petrel		$r = 0.36$, $F_{1,16} = 2.43$, $P = 0.14$
	cool season Mad. storm petrel		$r = 0.47$, $F_{1,25} = 7.07$, $P < 0.05$ $a = 5.9 \pm 0.7$ S.E., $b = -0.016 \pm 0.0061$ S.E.,
Down 1 + Down 2	Bulwer's petrel	$Hg = a*WL^b$	$a = 46.9$, $b = -0.221$, $r = 0.57$, $n = 26$
	Cory's shearwater		$a = 37.4$, $b = -0.501$, $r = 0.78$, $n = 88$
	Little shearwater		$a = 43.3$, $b = -0.619$, $r = 0.88$, $n = 16$
	hot season Mad. storm petrel		$a = 49.4$, $b = -0.467$, $r = 0.81$, $n = 26$
	cool season Mad. storm petrel		$a = 36.1$, $b = -0.477$, $r = 0.83$, $n = 44$
Breast feathers	Bulwer's petrel	$Hg = a + b*WL$	$r = 0.17$, $F_{1,15} = 0.46$, $P = 0.51$
	Cory's shearwater		$r = 0.46$, $F_{1,60} = 16.32$, $P < 0.0005$ $a = 1.2 \pm 0.1$ S.E., $b = 1.6E\text{-}3 \pm 4.0E\text{-}4$ S.E.,
	Little shearwater		not tested ($n = 5$)
	hot season Mad. storm petrel		$r = 0.033$, $F_{1,16} = 0.018$, $P = 0.90$
	cool season Mad. storm petrel		$r = 0.14$, $F_{1,26} = 0.49$, $P = 0.49$

TABLE 7.12. - Models and parameters for the regression between mercury concentrations (Hg) in plumage and age expressed as wing length (WL) for chicks of Laridae.

Plumage type	Species	Model	Regression parameters
Down	Yellow-legged gull	$Hg = a + b*WL$	$r = 0.29$, $F_{1,34} = 3.13$, $P = 0.086$
	Roseate tern	$Hg = a + b*WL$	$r = 0.67$, $F_{1,25} = 20.06$, $P < 0.0005$ $a = 3.8 \pm 0.2$ S.E., $b = -0.021 \pm 4.8E\text{-}3$ S.E.,
	Common tern	$Hg = a + b*WL$	$r = 0.45$, $F_{1,36} = 9.10$, $P < 0.005$ $a = 4.7 \pm 0.4$ S.E., $b = 0.022 \pm 7.3E\text{-}3$ S.E.,
Breast/flank	Yellow legged gull	$Hg = a + b*WL$	$r = 0.31$, $F_{1,33} = 3.43$, $P = 0.073$[a]
	Roseate tern	$Hg = a + b*WL$	$r = 0.66$, $F_{1,67} = 50.82$, $P < 0.0001$ $a = 2.6 \pm 0.2$ S.E., $b = 0.010 \pm 1.4E\text{-}3$ S.E.,
	Common tern	$Hg = a + b*WL$	$r = 0.58$, $F_{1,62} = 31.91$, $P < 0.0001$ $a = 2.6 \pm 0.2$ S.E., $b = 7.3E\text{-}3 \pm 1.3E\text{-}3$ S.E.,
Down + Breast/flank	Yellow legged gull	$Hg = a + b*WL$	$r = 0.077$, $F_{1,69} = 0.41$, $P = 0.52$
	Roseate tern	$Hg = a*WL^b$	$a = 14.0$, $b = -0.473$, $r = 0.82$, $n = 87$
	Common tern	$Hg = a*WL^b$	$a = 18.5$, $b = -0.481$, $r = 0.78$, $n = 94$

[a] the regression becames significant with the deletion of two extreme values:
$a = 3.0 \pm 0.4$ S.E., $b = -3.2E\text{-}3 \pm 1.5E\text{-}3$ S.E., $r = 0.35$, $F_{1,31} = 4.33$, $P < 0.05$.

Fig. 7.9
Variation of mercury concentrations in blood and body mass with age (expressed as wing lenght) of Cory's shearwater chicks.



legged gull chicks constitute an exception, although the deletion of two extreme mercury concentrations in breast/flank (probably from chicks with parents specialising in killing small Procellariiformes; Sub-chapter 2.1) produced a significant regression with negative slope (see Table 7.12).

The hypothesis that the decline of mercury concentrations in chick plumage is related to a growth dilution effect (Monteiro & Furness 1995) was tested with Cory's shearwater chicks by comparing the variations of mercury concentrations in blood and body mass with age (expressed as wing length) (Fig. 7.9). Both blood concentrations (BL) and body mass (BM) are strongly correlated with age (WL) ($r = 0.83$ and $r = 0.92$, respectively; $n = 63$) following two approximately symmetric quadratic curves:

$$BL = 0.979 - 8.52E\text{-}3 * WL + 1.89E\text{-}5 * WL^2 \quad (7.8)$$

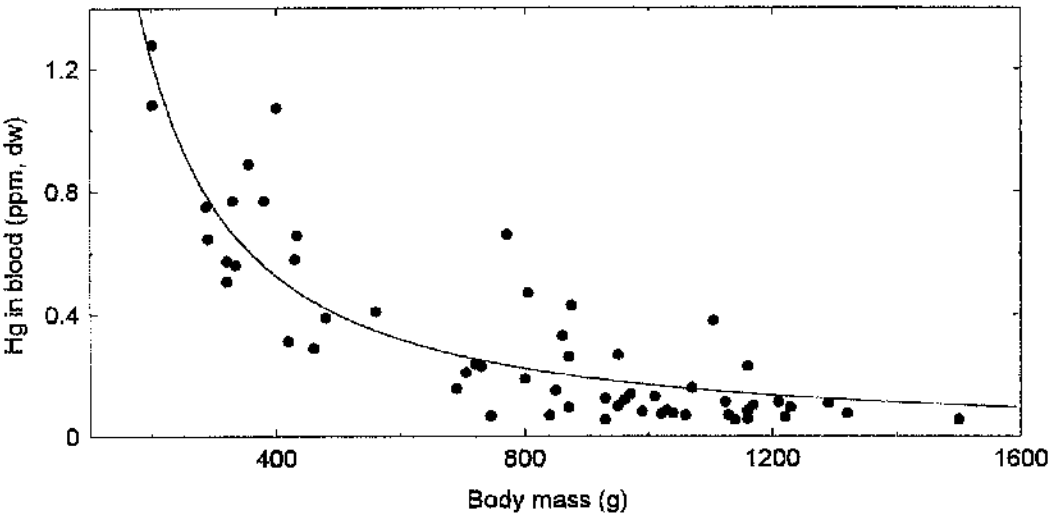
$$BM = 18.6 + 9.21 * WL - 1.86E\text{-}2 * WL^2 \quad (7.9)$$

Furthermore, there is a strong negative relationship between mercury concentrations in blood (BL) and body mass (BM) for the chicks used above ($r = 0.88$, $n = 63$; Fig. 7.10):

$$BL = 809.2 * BM^{-1.225} \quad (7.10)$$

and that confirms the existence of growth dilution of mercury concentrations in nestling tissues.

Fig. 7.10
Relationship between mercury concentrations in blood with body mass of Cory's shearwater chicks.



7.4. DISCUSSION

7.4.1. INTER-TISSUE VARIATION

Whereas the validity of inter-tissue ratios of mercury concentrations to infer levels in one tissue from concentrations measured in another was successfully challenged by Thompson *et al.* (1990), such ratios still help in understanding mercury dynamics in seabirds and potential influences of taxonomy. In this study, mercury concentrations showed important variations among the seabird tissues analysed and some general patterns were inferred from inter-tissue ratios of average mercury concentrations: 1) In adults the highest concentrations were observed in the plumage; 2) In chicks' plumage the concentrations were higher at hatching (down) than at fledging (breast); 3) Concentrations in hatchling's down were higher than in eggs; 4) Concentrations in adults' blood at laying and concentrations in eggs followed a consistent ratio around 1.4 (assessed only in Procellariiformes); 5) Ratios of concentrations adult:chick breast feathers and hatchling down:chick breast feathers were markedly higher in species with chicks possessing two coats of down than in species with a single coat. In contrast, some inter-tissue ratios did not follow patterns: 1) Concentrations in breast feathers of adults varied from higher to lower than concentrations in down of hatchlings both between and within taxonomic groups; 2) Although concentrations

in adults' breast feathers and blood followed a consistent ratio around 2.2 among four Procellariiformes, two atypical ratios presumably arising from major seasonal variations in blood levels in relation to moult (see below) prevented an assertion of a possible pattern.

Whereas most inter-tissue ratios (e.g. adult breast:blood, hatchling down:egg) appear to reflect to some extent hypothesised taxonomic-specific differences in mercury dynamics (Walsh 1990, Monteiro & Furness 1995) the evidence provided is still weak. This derived mainly from 'atypical' ratios from the cool season Madeiran storm petrel but also the Little shearwater, among the Procellariiformes, and some ratios from the Yellow-legged gull, among the Laridae. Moreover, inter-tissue mercury ratios were inconsistent between seasonal populations of Madeiran storm petrel and this probably arises from marked seasonal differences in diet as indicated by differences in average values of mercury concentrations (Sub-chapter 8.2). The stronger taxonomic-related difference relates with the number of coats of down in chicks and its effect on mercury dynamics in young seabirds (see below). This and other apparent taxonomic-dependent differences of mercury dynamics in seabirds (Sub-chapter 8.2) are best explained by marked differences in life histories, namely clutch size, patterns of chick growth, plumage development and adult moult, and feeding ecology, instead of presumed physiological differences in the handling of mercury. Elucidation of potential taxonomic influences on mercury dynamics in seabirds will require extensive comparisons of concentration ratios between tissues with a close ontogenic relationship (e.g. adult blood at laying : egg : hatchling down) and inter-tissue speciation of mercury.

7.4.2. Intra-tissue variation

7.4.2.1. Eggs

The information on mercury distribution among egg components is scant. It accumulates principally in the white proteins, bound to disulphide linkages within the ovalbumin fraction (Magat & Sell 1979, Takatera & Watanabe 1993), and does not accumulate in the eggshell (Burger 1994). These results confirm the albumen as the main store for mercury in bird eggs, with 80 to 90% of the egg contents' burden, and its absence from bird egg shells. However, this might not be a general pattern among vertebrates, since in sea turtle eggs more than 90% of mercury accumulates in the yolk (Sakai *et al.* 1995).

These results indicate that mercury concentrations are not influenced by the stage of incubation and provide a weak suggestion that rotten (possibly infertile) eggs may show extremely low concentrations possibly arising from reduced mercury deposition in eggs of abnormal composition. Nevertheless, Ohlendorf *et al.* (1988) and Becker *et al.* (1993b) did not find significant differences in mercury concentrations among eggs of different condition (incubated, failed to hatch or abandoned) and this needs more research.

7.4.2.2. Adults

A conceptual model for mercury dynamics in adult seabirds is required for the elucidation of most intra-tissue variation of mercury concentrations in plumage and blood samples observed in this study. A compartment model approach involves (Chapter 3: Fig. 3.1): ingestion from diet, uptake in the intestine, transport in blood, reversible accumulation in internal tissues, and elimination in eggs (only females), feathers and excreta. Therefore, a highly dynamic equilibrium might be expected between concentrations in feathers and in blood during feather development, with concentrations in blood depending on the body's burden as well as on the actual dietary intake (Furness *et al.* 1986, Braune & Gaskin 1987). Assuming a constant rate of mercury intake through the diet, higher blood levels are predicted at the start of moult, when mercury accumulated in soft tissues between moults starts to be mobilised into growing feathers, followed by a subsequent lowering of blood levels as the internal body pool of mercury diminishes.

The validity of such a model is supported indirectly by observations of declines of mercury concentrations along moulting feather sequences such as primaries (e.g. Furness *et al.* 1986) and in internal tissues with the progress of moult (Braune & Gaskin 1987, Stewart *et al.* 1994), but direct evidence from actual mercury concentrations in blood is presented for the first time in this study. First, from the decline of blood levels with progress of primary moult in Cory's shearwater (see Fig. 7.4). Secondly, from the strict resemblance of seasonal patterns of mercury concentration decline in blood and in growing breast feathers of Cory's shearwater (see Table 7.9 and Figs. 7.1B and 7.2). Furthermore, figure 7.3 illustrates the strong dependence of plumage mercury concentrations on those in blood at the time of feather formation. The pattern of seasonal decline in blood concentrations (Fig. 7.1B) also harmonises with the moult phenology of Cory's shearwater (i.e. a gradual onset of body moult during May-June that intensifies

after July and overlaps with tail moult in August and wing moult in September; Chapter 6: Fig. 6.2).

According to the compartment model, most variations of mercury concentrations in adult plumage might be accounted for by a moult effect (assuming a constant intake of mercury through diet). Hence, decreasing blood levels across the breeding season during moult are the clue for explaining general declines in mercury concentrations along primary sequences in regular primary moulting species (e.g. Furness *et al.* 1986, Honda *et al.* 1986) and in types of contour feathers moulting sequentially as in Cory's shearwater. This is supported by results on variation of concentrations between types of contour feathers. Firstly, there is a tendency for higher values in breast feathers (the first to be renewed, shortly after hatching; Sub-chapter 2.2: Fig. 2.5) than in back (renewed later) feathers. Secondly, breast and back feathers growing simultaneously on Cory's shearwater showed identical concentrations.

Seasonal variation of mercury loads and concentrations in tissues within seabird populations remains largely unknown (review in Monteiro & Furness 1995) and significant effects refer to decreases in internal tissue levels (Braune & Gaskin 1987, Stewart *et al.* 1994) and variations of blood and breast feather mercury concentrations (this study). While seasonal declines of concentrations in internal tissues and blood are associated with the progress of moult and result from mercury elimination to the plumage (Stewart *et al.* 1994, this study, Chapter 9), the origin of seasonal variations in breast feathers is less clear. Reversibility of mercury loads in formed feathers during the study period is ruled out, as mercury only incorporates in the feather while it is in formation (Crewther *et al.* 1965, Chapter 9) and then binds stably (Appelquist *et al.* 1984). A possible explanation is that the average mercury burden in breast feathers would vary in relation to a fluctuating age of breast feathers sampled each month. This hypothesis introduces again a moult effect and it was tested against information on moult phenology (Chapter 6: Fig. 6.2) and seasonal variation of blood and breast feather mercury concentrations (this Chapter: Fig. 7.1) available for Cory's shearwater. As the full development of a breast feather requires nearly 2 months (Monteiro unpubl. data), lower breast feather concentrations in early July (i.e. at hatching and when breast moult intensifies) are explained by the poorer representation of the older feathers shed firstly and with higher mercury loads; increasing concentrations from July to September result from an increasing proportion of newly formed feathers with high mercury loads; intermediate and constant concentrations from March to May

reflect a invariable breast plumage during a period of no moult. This might be a more general phenomenon but the poor knowledge of moult patterns or small sample sizes preclude hypothesis testing with the other study species. The observed variation in breast feather concentrations within and between seasonal populations of Madeiran storm petrel is complex and may constitute a case where a presumable moult effect is masked by seasonal differences in diet (Sub-chapter 8.2).

Although moult is the explanatory factor for most of the observed intra-tissue variability of mercury concentrations in adults, the contributions of other potential factors were also tested. Similar mercury concentrations observed in breast (white) and back (dark brown) feathers developing simultaneously in Cory's shearwater confirm that mercury concentration is not influenced by feather melanin content as shown by Burger & Gochfeld (1992). Mercury concentrations appear be dependent on the developmental stage of feathers (e.g. breast, primaries, secondaries or rectrices) collected during natural or induced moult. Partly-grown feathers tend to show higher mercury concentrations than fully-grown feathers in overall comparisons (Walsh 1993, Burger *et al.* 1992) and in some cases within the same individuals (this study). The most likely explanation for this is that such differences may reflect seasonal declining levels of blood mercury concentrations during moult and associated higher levels in the firstly grown feathers, although differences in mercury in diet at the time of feather formation have been hypothesised (Burger *et al.* 1992).

Egg production offers an additional route for mercury elimination in females and potentially leads to differences in mercury dynamics and concentrations between sexes (review in Monteiro & Furness). However, the evidence for inter-sex differences in seabirds is limited to slight lowering of concentrations in females in particular feather types (Braune & Gaskin 1987, Lewis *et al.* 1993, Stewart *et al.* 1994). Our results confirm such a tendency, with average mercury concentrations in breast feathers and blood of female Cory's shearwater being respectively 17% and 13% lower in females. Moreover, an inter-sex comparison of monthly blood mercury concentrations might provided further insights into sex-related differences in mercury dynamics (assuming a constant proportion of dietary intake of mercury over the whole breeding season between sexes). Mercury elimination in females via the egg explains the 14% increase in the ratio of blood concentrations male:female from May to June (laying occurs in late May; Sub-chapter 2.2) but more pronounced increases in this ratio observed in August-

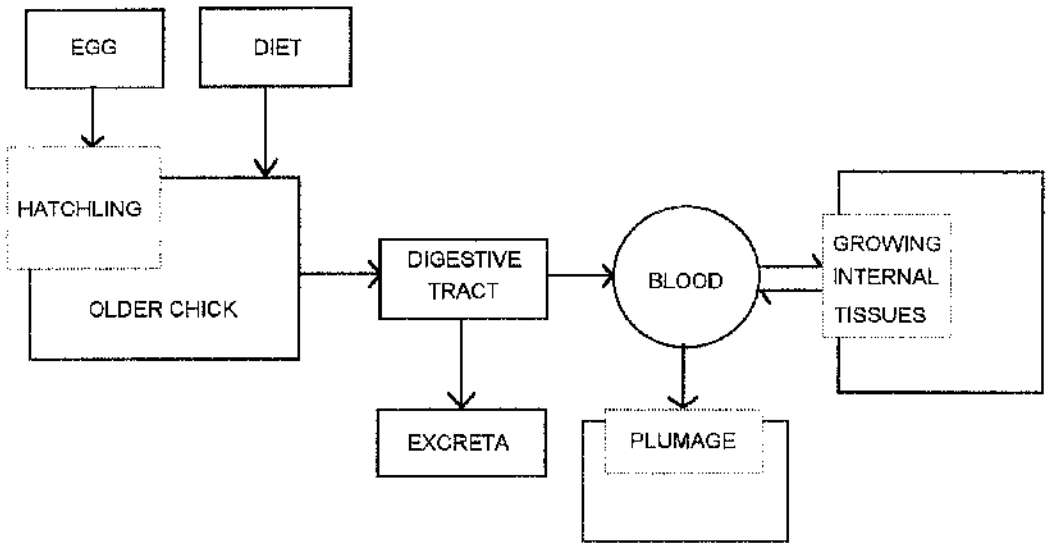
September are best explained by presumed but poorly understood inter-sex differences in moult phenology (Chapter 6). In Cory's shearwater the clutch is a relatively minor mercury sink, with about 35µg of mercury being eliminated into the single egg (basis: concentration of 0.4 ppm fw and 85g egg content) against an estimated 400µg excreted into the plumage (basis: 5 ppm fw concentration and plumage with 10% of a 800g body mass). This explains the lack of major inter-sex differences in fluctuations of mercury concentrations in blood and internal tissues over the breeding season.

Investigations into age-related changes of mercury concentrations in tissues of adult seabirds have been few in number and generally limited to body feathers (Furness *et al.* 1990, Thompson *et al.* 1991, 1993, Burger *et al.* 1994). All demonstrated that mercury does not accumulate with adult age and this is attributed to a balance between metal dietary intake and elimination to the plumage at each moult preventing long-term accumulation in the body with increasing age. Although age effects in tissues of adults were not investigated thoroughly in this study, we collected circumstantial evidence of non-accumulation with age from the similarity of mercury concentrations in samples of breast feathers obtained from the same individuals, at the same month, in subsequent breeding seasons. Moreover, pairs of mercury concentrations in individual birds in year *n* and year *n* plus *1* to *3* show a general tendency to be correlated, though larger sample sizes are required to ascertain this accurately for most species due to important intra-sample variability of mercury concentrations within feather samples (Walsh 1993, Chapter 4). Thus, at least in some taxa individuals exhibit consistently 'low' or 'high' mercury concentrations between seasons and this suggests that mercury burdens are an intrinsic characteristic possibly resulting from individual dietary specializations and physiological differences.

7.4.2.3. Chicks

A conceptual model for mercury dynamics in young seabirds is required for the elucidation of most intra-tissue variation of mercury concentrations in plumage and blood samples observed in this study. A modified version of a compartment model developed for adults (Monteiro & Furness 1995) is presented in figure 7.11 and involves: heritage from egg, ingestion from diet, uptake in the intestine, transport in blood with simultaneous accumulation in growing internal tissues and developing plumage and elimination in excreta. Therefore, a highly transient process is expected, as concentrations in blood will depend on a balance between

Fig. 7.11
Compartment model for mercury dynamics in young seabirds.



mercury intake through the diet and accumulation in the rapidly growing plumage and internal tissues. Altogether, the model introduces two main differences to that proposed for adults. First, the egg-derived origin of mercury in hatchlings plumage. Second, a short-term exceptional growth of chicks (i.e. 3-12 weeks cf. adult constant body size), accompanied by an entire plumage formation (equivalent to a complete adult moult cycle) and a 10 fold increase in body mass.

The validity of the former model is supported by the literature and by the results from this study. The hypothesis that hatchlings' body burdens of mercury are determined mostly by the levels in the egg (Becker *et al.* 1993a,1994) is confirmed by significant correlations between concentrations in females' blood at laying and concentrations in down of hatchlings (this study), and by experimentally established dose-response relationships between concentrations in females' blood and in eggs (Chapter 9). Furthermore, highly transient mercury concentrations in blood of Cory's shearwater chicks illustrate a predicted growth dilution effect (Monteiro & Furness 1995) resulting from an imbalance between mercury dietary intake and co-accumulation in developing plumage and internal tissues.

Information on variation of mercury concentrations among plumage types of chicks is scanty (Monteiro & Furness 1995) but extensive variation of mercury concentrations between and within chick plumage types is reported here. The results show a general trend of marked decline in mercury concentrations across

the successive types of plumage developed during chick growth, i.e. down1, down2 and definitive plumage. Such higher mercury concentrations in down grown in the egg than in contour feathers grown later, along with significant negative correlations of concentrations with age both in single or pooled plumage types, provide evidence of a generalised growth dilution effect in most of the study-species. However, this is not a general pattern and several cases were reported where plumage mercury concentrations were found to be independent of chick's age or even to increase with age (Thompson *et al.* 1991, Becker *et al.* 1993a, Burger *et al.* 1994).

Linear blood-feather relationships of mercury concentrations in chicks and adults obtained at different orders of magnitude of blood concentrations (i.e. 0.05-0.3 ppm dw and 0.5-5 ppm dw, respectively) show consistent slopes in the range 2.3-3.4. Presuming that such slopes represent an inter-tissues partition coefficient, their resemblance advocates the existence of a standard partition coefficient between growing feather and blood in the order of 3. Therefore, differences in mercury concentrations among plumage types of chicks cannot be a consequence of saturation of metal-accumulatory ability. That strengthens the growth dilution effect as the best explanatory hypothesis for age-related mercury declines in chick plumage.

7.4.3. Implications for monitoring

The need to consider inter- and intra-tissue mercury variability in avian indicator species when designing and interpreting monitoring studies of mercury has been reviewed extensively (Furness 1993, Monteiro & Furness 1995) and it is strengthened further by findings in this study. Besides aspects already addressed in those recent reviews, several key-points raised here are highlighted below.

1. Assuming a current intra-tissue variation of mercury concentrations representing about 30% of the mean and following Zar (1984), minimum sample sizes of 108 or 27 for each of two populations are required to achieve a 90% probability of detecting, respectively, 10% or 20% differences between two population means, using t-tests.
2. Differences in site-specificity of mercury among egg components and possible constraints in mercury deposition into infertile eggs make advisable the use of whole contents from intact fertile eggs as monitoring unit.

3. Blood mercury concentrations show wide short-term variations in relation to moult and therefore are of limited interest for spatial and temporal monitoring.
4. When using adult contour feathers for monitoring, moult should always be considered as it is a main potential confounding factor responsible for most variation of mercury concentrations in adults' plumage.
5. Other confounding factors should be considered, in relation with moult, when using adult contour feathers for monitoring: type of feather, developmental stage of feathers, sex and season.
6. Plumage mercury concentrations may vary markedly between adult and juvenile birds and it is therefore important to avoid sampling from a mixture of these classes, especially in Procellariiformes where adults and juveniles are closely similar in plumage.
7. Chick plumage should be used with caution for monitoring, since different plumage types may produce dramatically different indications of mercury levels in seabird populations.
8. Potential age-related variations of mercury concentrations in chick's plumage need to be considered in monitoring. They might be controlled with careful sampling design and multivariate comparisons of concentration-age relationships (e.g. via analyses of covariance).
9. Uneven and poorly understood patterns of mercury variation in adults and young of seasonal populations of Madeiran storm petrel emphasise the need for a detailed knowledge of the biology and ecology of indicator species.

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CHAPTER 8

MERCURY ACCUMULATION IN PREY FISH AND INTER-SPECIFIC VARIATION OF MERCURY BURDENS IN SEABIRDS

CONTENTS

Sub-chapter 8.1

MERCURY CONCENTRATIONS IN PREY FISH INDICATE ENHANCED BIOACCUMULATION IN MESOPELAGIC ENVIRONMENTS

8.1.1. INTRODUCTION

For a number of reasons, mercury and its biogeochemical cycle are unique among metals of concern for their potential harmful environmental effects. Mercury evaporates to the atmosphere due to a high vapour pressure and forms strong covalent bonds in biological systems (e.g. Lindberg 1987, WHO 1990). Unlike most other toxic metals, inorganic mercury is efficiently biotransformed into an organic form (methylmercury) in several compartments of the aquatic environment, including the water column (Topping & Davies 1981).

Mercury bioaccumulates in aquatic organisms, i.e. the concentrations in the organism tissues increase to a high dynamic equilibrium or even increase throughout the life span. In addition, mercury is the only metal that consistently biomagnifies through the food chain, i.e. predators accumulate higher tissue concentrations than in their food. Although the factors controlling the accumulation of mercury in aquatic organisms are poorly understood, it is widely recognised that the accumulation of methylmercury from food or seawater is much greater than that of inorganic mercury (e.g. Boudou & Ribeyre 1985, Canli & Furness 1995).

Recent advances in knowledge of distribution and speciation of mercury in oceanic waters showed increased concentrations of methylmercury in seawater below the thermocline (Mason & Fitzgerald 1990, Cossa *et al.* 1994) as a consequence of microbial mediated methylation of reactive mercury supplied by scavenging of particulate mercury from the mixed-layer (Mason & Fitzgerald 1993). Thus, enhanced bioaccumulation of mercury by organisms in such low oxygen environments has been predicted. To test this hypothesis, mercury concentrations were analysed in eight short-lived fish species of low trophic level (essentially second order consumers) and related to their vertical distribution.

8.1.2. MATERIALS AND METHODS

Fish species selected were some of the most common prey species in the local food webs and cover a wide range of vertical distributions, from epipelagic to mesopelagic environments. All samples were collected in the Azores archipelago and obtained from the fish collection at the University of the Azores (Department of Oceanography and Fisheries), fishing boats and fish dropped at seabird colonies. Details of sampling periods and methods of preservation, together with information

on food and vertical distribution are given in Table 8.1.1.

For each fish, total length was measured with a ruler (to 0.1 cm) prior to dehydration to a constant weight in an oven at 50°C. Homogenised samples of individual whole fish were analysed for total mercury. When concentrations in individual fish were below detection limits, pooled samples of similar sized fish were used. Sample pre-treatments, total mercury determination, analytical control and data analysis follow the procedures described in Chapter 4. Recoveries of inorganic mercury averaged 79.2% (S.E. = 2.7, n = 18) and all concentrations were corrected by this factor. Hereafter mercury refers to total mercury and all values are given in nanograms per gram on a dry weight basis (ppb, dw). Concentrations may be converted to a fresh weight basis using as reference the overall average percentage of moisture of 68.8% (S.E. = 0.5%, n = 95) or species-specific values given in Chapter 4.

Potential bias in mercury concentrations related with method of preservation was assessed by assigning randomly 30 fresh *Macroramphosus scolopax* of standard size (7-9 cm) to three treatment groups (n = 10 each) during 8 weeks: (1) frozen at -20°C; (2) formaldehyde at 5%; (3) alcohol at 70%. The average mercury concentrations in the three treatment groups (mean $\pm$S.E., ppb dw: (1) 46 $\pm$8, (2) 53 $\pm$3.2, (3) 60 $\pm$2.8) were not significantly different (1-way ANOVA, $F_{1,27} = 2.35$, $P = 0.11$).

8.1.3. RESULTS

Average mercury concentrations in the eight study-species ranged from 57 to 377 ppb dw (Table 8.1.2). Average mercury concentrations and median daytime depth (Fig. 8.2.1) show a significant positive correlation ($r_s = 0.87$, $t_6 = 4.41$, $P < 0.005$). Concentrations increase by four-fold from epipelagic (<100m) to mesopelagic (>300 m) species. Intermediate concentrations were observed in species occurring in the transition (median depths of 200-250m) between epipelagic and mesopelagic environments. Concentrations did not vary among mesopelagic species (Kruskal-Wallis, $H_{3,n=41} = 2.08$, $P = 0.56$).

An increase in mercury concentrations with depth is illustrated at the species level in *Capros aper* (Fig. 8.1.2). The second half of the size range, which occurs typically below 200m (S. Holzlöhner & A. Orlowski, pers. com.), showed concentrations typical of mesopelagic species while the first half showed concentrations typical of epipelagic species.

TABLE 8.1.1. - Sampling and conservation details plus ecological characteristics (source UNESCO 1986) of small epipelagic and mesopelagic fish used in this study.

Species	Sampling period	Method of preservation	Food	Vertical distribution	Median daytime depth (m)
Macroramphosus scolopax	Aug, Nov 1993 Jul 1995	frozen alcohol	invertebrates, mainly copepods	juvenile (<10 cm) epipelagic adults benthic, 50-150m	100
Scomber scombrus	Apr 1995	frozen	chiefly copepods and euphausiids	epipelagic or meso-demersal to 200m	100
Capros aper	Oct-Dec 1990, 1994 Jul 1995	frozen alcohol	crustaceans (mainly copepods), molluscs	benthic, 40-600m, mainly 100-400m	250
Trachurus picturatus	Apr, May 1995	frozen	crustaceans	pelagic-demersal down to 370m	200
Maurolicus muelleri	Sep 1979	alcohol *	copepods and euphausiids	mesopelagic: day, 200-400m night, into upper 100m	300
Electrona rissoi	Jul 1995	alcohol	no data	mesopelagic: day, 225-700m night, 90-375m	500
Myctophum punctatum	Jul 1994	alcohol *	copepods and euphausiids	mesopelagic: day, 700-1000m	800
Ceratoscopelus maderensis	Oct 1978	alcohol *	copepods and other planktonic crustaceans	mesopelagic: day, 900-1500m night, 25-200m	1200

* kept initially in formaldehyde at 5% prior to final preservation in alcohol at 70%.

TABLE 8.1.2. - Total length and total mercury concentrations for the small epipelagic and mesopelagic fish samples used in this study. Length and mercury values are mean ±S.E. and range below.

Species	n	Total length (cm)	Hg (ppb, dw)
Macroramphosus scolopax	42	8.0 ±0.2 5.7-11.4	57 ±3 14-115
Scomber scombrus	4	26.0 ±1.7 21.5-28.5	91 ±12 71-122
Capros aper	19	8.7 ±0.6 4.6-13.0	147 ±24 32-331
Trachrus picturatus	20	16.8 ±0.7 9.8-21.7	149 ±27 26-469
Maurolicus muelleri	11	4.6 ±0.1 4.1-5.2	343 ±23 251-446
Electrona rissoi	10	8.1 ±0.3 6.8-9.0	323 ±45 145-533
Myctophum punctatum	6	7.7 ±0.2 7.0-8.3	320 ±35 150-367
Ceratoscopelus maderensis	14	6.8 ±0.1 6.5-7.5	377 ±9 318-423

8.1.4. DISCUSSION

Because of human health concerns (Clarkson 1990), mercury accumulation in commercial predatory fish has been studied thoroughly and concentrations have been found to increase with species trophic level and longevity, as well as fish age (e.g. Riisgård & Hansen 1990, Monteiro *et al.* 1991, Joiris *et al.* 1995). Information on mercury accumulation in non-commercial small prey fish of low trophic level is scanty. Results from this study indicate that vertical distribution is a major factor contributing to inter-specific and *C. aper* intra-specific variations in mercury levels. Age-dependent accumulation of mercury in these extremely short-lived species (less than 2 years; e.g. Linkowski *et al.* 1993) is unlikely to account for the observed fourfold increase in mercury concentrations between the epipelagic and mesopelagic fish species. Thus, this increase seems to arise from

Fig. 8.1.1
Relationship between mercury concentrations and median daytime depth for eight fish species in the Azores. Species codes are the initials of scientific names.

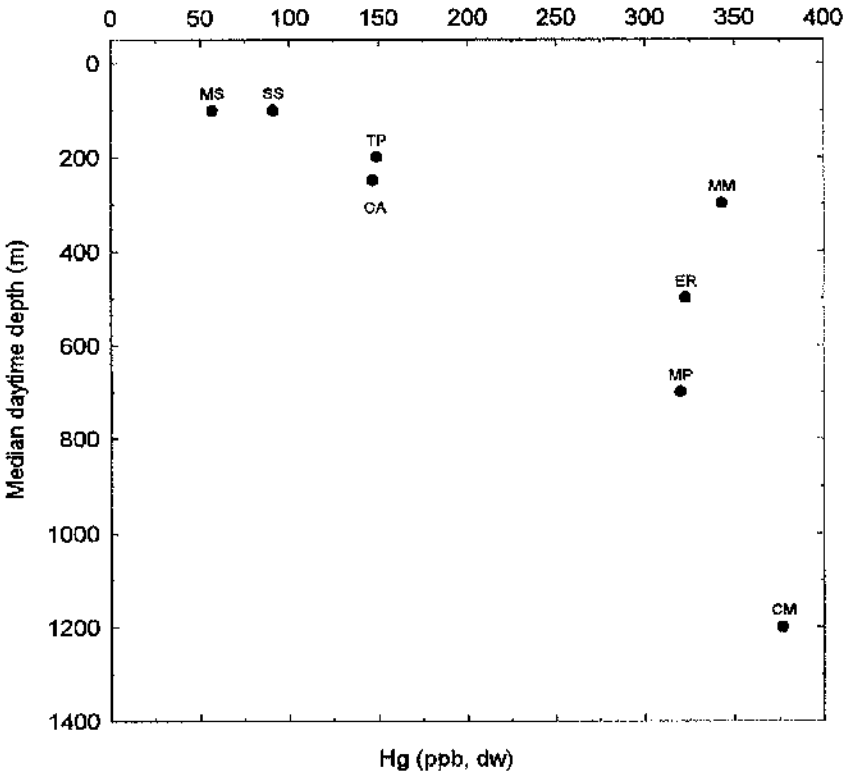
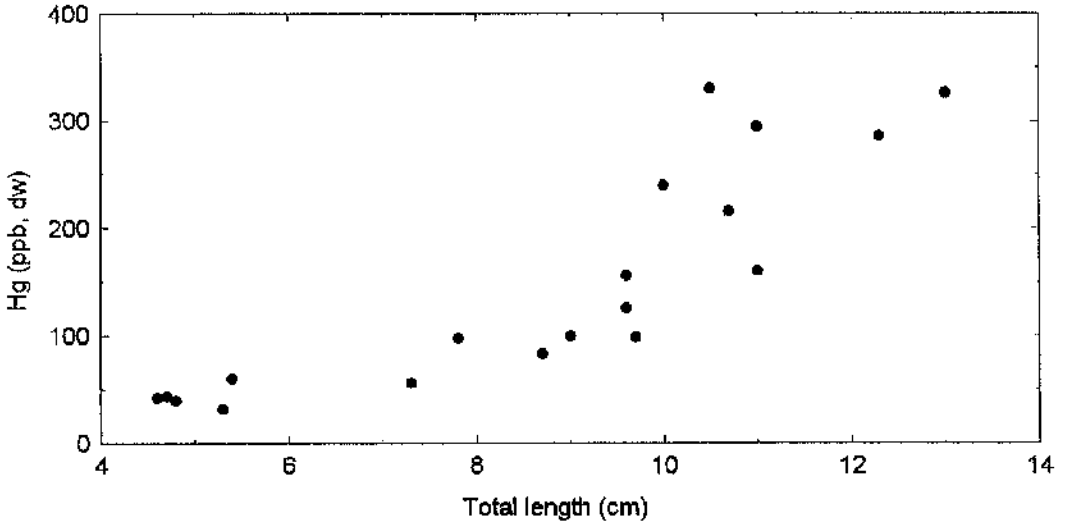


Figure 8.1.2
Relationship between mercury concentrations and total length in Capros aper from the Azores.



the remarkably elevated availability of methylmercury in sub-thermocline waters (depths > 200m in Atlantic and Pacific and depths > 100m in Mediterranean; Mason & Fitzgerald 1993, Cossa *et al.* 1994, Mason *et al.* 1995). Moreover, uniform mercury concentrations observed among mesopelagic fish species with varying depth ranges (300-1200m; this study) are consistent with absence of major variations of methylmercury concentrations in sub-thermocline ocean waters (Mason & Fitzgerald 1990, Cossa *et al.* 1994, Mason *et al.* 1995).

Results from this study confirm the predicted enhanced methylmercury bioaccumulation in sub-thermocline low oxygen waters. This begs an appraisal of the relative importance of uptake from food or water in this environment. The biomagnification of mercury resembles that of hydrophobic organic trace pollutants rather than that of ionic metals and it is generally thought to result from the lipid solubility of methylmercury. This seems an inadequate explanation for two reasons: (1) unlike other hydrophobic compounds, methylmercury in fish resides in protein rather than fat tissue (Bloom 1992); (2) neutral complexes of inorganic mercury are as soluble as their methylmercury analogues and accumulate passively in phytoplankton (Mason *et al.* 1995). However, differences in partitioning within phytoplankton cells between inorganic (which is principally membrane bound) and methylmercury (which accumulates in the cytoplasm) lead to a greater assimilation of methylmercury during zooplankton grazing (Mason *et al.* 1995). Thus, most of the discrimination between inorganic and methylmercury seems to occur during trophic transfer at the base of the food chain while the major enrichment factor is between water and phytoplankton (Mason *et al.* 1995). As a result, enhanced methylmercury bioaccumulation in fish in sub-thermocline waters would be determined proximately by levels in food and ultimately by water chemistry which controls methylmercury speciation and uptake at the base of the food chain. This is supported by the much higher efficiency of methylmercury uptake from ingested food (70%) than from water passed over the gills (10%; Phillips & Buller 1978).

Enhanced bioaccumulation of mercury in mesopelagic organisms of low trophic level arising from sub-thermocline reservoirs of methylmercury (this study) is the best explanation for high and yet poorly understood mercury concentrations found in deep-sea predators (e.g. Phillips 1980). This poses an additional problem regarding the human and environmental health risks of mercury (e.g. WHO 1990, Fitzgerald & Clarkson 1991) due to the increasing importance of deep-sea marine organisms as source of protein for humans (Pitchner & Hart 1987). Besides, the

global increases in mercury contamination over the last century (Mason *et al.* 1994) is affecting the marine ecosystems (Slemr & Langer 1992, Thompson *et al.* 1992, Rolfhus & Fitzgerald 1995) and were amplified in mesopelagic environments (Sub-chapter 11.2).

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Sub-chapter 8.2

THE RELATIONSHIP BETWEEN MERCURY BURDENS AND DIET IN SEABIRDS FROM THE AZORES

8.2.1. INTRODUCTION

The existence of large variations in mercury burdens among seabird species has been attributed to a variety of proximate and ultimate factors such as trophic level, migratory habits, body size, life span, moult pattern and taxonomic influences on physiology (Walsh 1990, Monteiro & Furness 1995).

Dietary and feeding differences have been proposed as the best explanation for consistently low mercury burdens in those species which feed predominantly upon crustaceans compared to burdens in fish- and squid-eating species (Braune 1987, Honda *et al.* 1990, Lock *et al.* 1992). Moult-related constraints in mercury elimination to the plumage seem to increase mercury burdens in species with longer than annual moult cycles, like some albatrosses (Furness *et al.* 1986, Honda *et al.* 1990). Migratory habits might account for mercury burdens in populations subjected to major differences in dietary mercury between breeding and non-breeding grounds (Leonzio *et al.* 1986). Life-span seems to have an irrelevant contribution to mercury burdens of adult in species so far studied, as age-related variations are lacking (review in Monteiro & Furness 1995). Potential taxonomic influences on physiology and mercury dynamics seem to be unimportant in determining seabirds' mercury burdens (Lock *et al.* 1992, Chapter 7).

Besides the known contributions of all the above factors, major inter-specific differences in mercury burdens among fish and squid eating species, especially Procellariiformes, are poorly understood (Muirhead & Furness 1988, Monteiro *et al.* 1995). Since enhanced bioaccumulation in mesopelagic organisms has been demonstrated (Sub-chapter 8.1), there is a rational to hypothesise that major inter-specific differences in mercury burdens among seabirds may be related to the relative importance of epipelagic and mesopelagic prey in their diet. To test this, I present here a comparison of mercury levels in diet, and body-burdens of fish- and squid-eating seabirds from the Azores.

8.2.2. MATERIALS AND METHODS

The study-seabirds were selected according to their predominant dietary and feeding characteristics (Sub-chapter 2.2, Prince & Morgan 1987) in order to represent two dichotomous groups, one exploiting mesopelagic prey -Bulwer's

petrel *Bulweria bulwerii* and the temporally segregated Hot and Cool season Madeiran storm petrel *Oceanodroma castro* populations (Chapter 5)-, and another exploiting epipelagic prey -Cory's shearwater *Calonectris diomedea borealis*, Little shearwater *Puffinus assimilis baroli* and Common tern *Sterna hirundo*. Breast feather samples are considered to provide the most reliable indication of the whole-plumage burden of mercury in adult birds (Furness *et al.* 1986, Chapter 7) and thus were used. Food samples, consisting of spontaneous or induced regurgitations (also pellets for Common tern), were collected for study of diet composition and determination of mercury concentrations. Induced regurgitations were obtained using a stomach-pump (for Cory's shearwater) or a 50ml seringe (for Little shearwater and the petrels), salt water and a catheter adequate to species size (Wilson 1984, Gales 1985).

Sampling was undertaken in the Azores archipelago between April 1993 and June 1995 at three multispecific seabird colonies (Vila, Praia and Baixo Islets), with the exception of some Common tern pellets collected in other Azorean colonies (Corvo and Topo islet). Food samples were obtained from adults during the chick rearing periods, except for some samples from Cory's shearwater collected in March and all samples from Little shearwater that were collected in September-October. After examination for the presence of fish, squid or crustaceans, the excess salt water was removed and all solid remains preserved in 70% alcohol for latter analysis. In the laboratory, the solid fraction was examined under a microscope and all diagnosing hard parts (otholits, vertebra, opercular, maxillary and pre-maxillary bones, *cleitra*, squid beaks, among others) were collected for identification of the prey species. The edible component (made mainly of muscle accounting for over 80% of the whole net mass) was dehydrated to a constant weight in an oven at 50°C, and homogenised prior to analysis for total mercury. Recoveries of inorganic mercury spikes in regurgitations averaged 69.3% (S.E.=6.7, n=8) and all concentrations were corrected accordingly. Potential bias of mercury concentrations related with method of preservation was assumed to be negligible based on the assessment reported in Sub-chapter 8.1.

For those aspects not mentioned here, sample collection, preparation and storage, total mercury analysis, analytical quality control, and data analysis followed the procedures described in Chapter 4. Hereafter mercury refers to total mercury and values are given in microgram per gram on a fresh weight basis for breast feather (ppm, fw) and nanogram per gram on a dry weight basis (ppb, dw) for food samples.

TABLE 8.2.1. - Diet composition for some seabirds at the Azores archipelago. Species codes are the initials of common names given in Table 8.2.2. Food origin is categorised as epipelagic (EP) or mesopelagic (MP). The frequency of occurrence of food types is scored: absent (0); 1-20% (1); 21-40% (2); >40% (3). Information derived from regurgitations for the Procellariiformes (Granadeiro & Monteiro unpubl. data) and from pellets for the common tern (Granadeiro *et al.* 1995).

Diet composition		Seabird species (n)					
Food type	Origin	BP (21)	HMSP (39)	CMSP (11)	CS (82)	LS (10)	CT (779)
Fish							
Macroramphosus scolapax	EP				3		3
Capros aper	EP-MP				2		2
Trachurus picturatus	EP-MP				1		2
Scomberesox saurus	EP				2		1
other epipelagic fish	EP				1	3	1
lanternfish	M	3	3	3	0	0	1
Cephalopods	EP,MP	1	1	1	2	1	0
Crustaceans	EP,MP	1	1	1	1	0	0

8.2.3. RESULTS

A summary of diet composition of the study-seabirds is shown in Table 8.2.1. These data validate the separation into two groups based on the predominant origin of their prey, with the petrels exploiting mesopelagic prey and the shearwaters and the tern exploiting epipelagic prey.

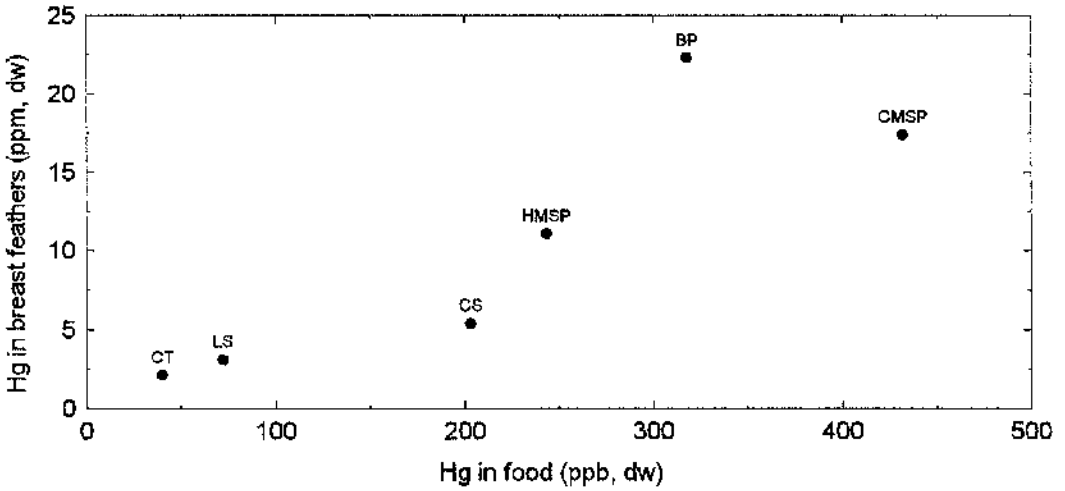
Mercury concentrations in regurgitations and breast feathers of the study-seabirds are shown in Table 8.2.2. A comparison of average mercury concentrations in food and breast feathers (Fig. 8.2.1) indicates a significant positive correlation between variables (Spearman rank order correlation, $r_s = 0.94$, $P < 0.005$, $n = 6$). A biomagnification factor for each species was derived from the ratio of average mercury concentrations in feathers (ppm, fw) and food (expressed in ppm, fw; based on a fraction of moisture in fish of 0.68, Chapter 4), with an overall average of 145 (S.E. = 18, n = 6).

TABLE 8.2.2. - Mercury concentrations in food (regurgitations) and breast feather samples of some seabirds from the Azores Archipelago. Values are mean ±S.E., sample size bracketed and range below.

Species	Food (ppb, dw)	Breast feathers[a] (ppm, fw)	Biomagnification factor[b]
Bulwer's petrel	318 ±47(15) 43-738	22.3 ±0.4 (91) 13.8-32.8	219
Hot season Madeiran storm petrel	243 ±37 (33) 22-879	11.1 ±0.3 (100) 5.4-23.0	143
Cool season Madeiran storm petrel	432 ±94 (9) 128-938[c]	17.4 ±0.4 (130) 6.8-34.3	126
Cory's shearwater	203 ±28 (32) 23-722	5.4 ±0.1 (186) 1.9-10.4	83
Little shearwater	72 ±33 (3) 27-136	3.1 ±0.1 (82) 1.5-6.9	135
Common tern	40 ±5 (6) 20-70	2.1 ±0.1 (27) 1.2-3.5	164

[a] Values from Chapter 7.
[b] Definition in text.
[c] Outlier of 2352 ppb dw excluded.

Figure 8.2.1
Comparison of mean mercury concentrations in food and breast feather samples for selected seabirds from the Azores. Species codes are the two initials from the common names in Table 8.2.2.



Mercury concentrations in food samples of Cory's shearwater were highly significantly different (t-test, $t_{30}=6.47$, $P<0.0001$) between the two months considered (mean $\pm$S.E.): March (328 $\pm$34, n=16) and August (80 $\pm$18, n=16). Mercury concentrations in food samples of the Hot season (samples from August) and the Cool season (samples from December and January) Madeiran storm petrel populations (see Table 8.2.2.) were significantly different (t-test, $t_{40}=2.20$, $P<0.05$).

8.2.4. DISCUSSION

This study provides the first field evidence of a direct relationship between dietary mercury and seabirds' body burdens. The inter-species variation of mercury body burdens is not attributable to differences in trophic level, as all the selected seabirds feed predominantly on fish and/or squid and are essentially third order consumers in their food chains. The operational link between mercury in diet and seabirds' body- burden appears to be the dichotomy arising from the predominance of epipelagic or mesopelagic prey in the diet. Indeed, average mercury concentrations in breast feathers of seabirds feeding predominantly on epipelagic prey range from 2.1 to 5.4 ppm fw while those of seabirds feeding predominantly on mesopelagic prey range from 11.1 to 22.3 ppm fw. Such enhanced bioaccumulation of mercury by seabirds specialised on mesopelagic prey matches closely the four-fold increase of mercury concentrations from epipelagic to mesopelagic prey fish (Sub-chapter 8.1). These findings suggest that high mercury burdens found in most fish and squid eating petrels and storm petrels (Muirhead & Furness 1988, Honda *et al.* 1990, Lock *et al.* 1992, Monteiro *et al.* 1995) derive mainly from specialisation in exploiting diel vertically migratory mesopelagic prey (Prince & Morgan 1987), though moult-related constraints in mercury elimination may also account for some cases (Muirhead & Furness 1988). Hence, feeding specialisation emerges as a major proximate source of inter-species variation of mercury burdens among fish/squid eating seabirds.

The large intra-specific seasonal differences in mercury concentrations of food samples analysed here are presumably a consequence, and simultaneously an indication, of important seasonal variations in diet composition. Indeed, substantial variations in diet composition were detected in Cory's shearwater regurgitations; in 1994, between March (n=52) and August (n=24) the frequencies of

occurrence of cephalopods and *Macroramphosus scolopax* varied, respectively, from 43 to 1% and 35 to 85% (Granadeiro unpubl. data). These two major seasonal variations in diet are likely to account for the corresponding decrease observed in dietary mercury concentrations (cf. Results), as *M. scolopax* exhibits typically low mercury levels (Sub-chapter 8.1) and squid preyed upon by Cory's shearwater in the North-east Atlantic include a significant proportion of mesopelagic species (M. Clarke pers. com.) with presumed enhanced mercury levels (cf. Sub-chapter 8.1). Dietary differences between the two temporally segregated populations of Madeiran storm petrel were suggested by different mercury burdens in eggs and chicks (Chapter 7) and are strengthened here by mercury concentrations of regurgitations from the two distinct chick rearing periods. Although the elucidation of this hypothesis is hampered by the lack of detailed dietary information for these populations, mercury concentrations in diet suggest variations in the relative importance of mesopelagic prey to the species diet throughout the year, which appear to be more important during the cooler period.

The findings from this study contribute new insights into the potential of seabirds as monitors for mercury. First, mercury biomagnification (i.e. increase with trophic levels) high in the food chains involves primarily bioaccumulation of methylmercury (Riisgård & Hansen 1990, Mason *et al.* 1995) and, in this respect, data presented here constitute the first quantitative field assessment of mercury enrichment associated with trophic transfer between fish/squid and seabirds. Assuming that virtually all mercury in fish and bird feathers is methylmercury (>95%; Bloom 1992, Thompson & Furness 1989), the ratios of average feather:food total mercury concentrations indicate an average enrichment factor in the order of 150x. This compares with typical enrichment factors of 2-10x observed in field studies with zooplankton and fish muscle, including top predators (Windom *et al.* 1976, Watras & Bloom 1993, Monteiro unpubl. data), emphasising further the value of seabirds as monitors for mercury and particularly of bird feathers as monitoring units (cf. Furness *et al.* 1986). Second, ecological segregation in seabird communities has resulted in many parts of the world into feeding specialisation on epipelagic or mesopelagic organisms (especially among Procellariiformes; Prince & Morgan 1987). This offers an unique opportunity for easy and inexpensive monitoring of current geographical and historical variations in mercury contamination within and between the epipelagic and mesopelagic compartments of the marine ecosystems (e.g. Chapter 11).

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CHAPTER 9

KINETICS, DOSE-RESPONSES, EXCRETION AND TOXICITY OF METHYLMERCURY IN FREE-LIVING ADULT CORY'S SHEARWATER

CONTENTS

9.1. INTRODUCTION

Widespread contamination of the ecosphere by mercury has been demonstrated in recent years (e.g. Mason *et al.* 1994, Chapter 11). One of the principal environmental health concerns regarding mercury pollution is neurotoxicity resulting from methylmercury ingestion both in humans (review in WHO 1990) and wildlife (e.g. Scheuhammer 1987). Mercury monitoring is thus a priority and biomonitors became popular because of their capacity to integrate contamination both from the abiotic and biotic compartments of the ecosystems over convenient spatial and temporal scales (e.g. Phillips 1980).

Avian studies of mercury have multiplied since the 1960s, focusing on the toxicokinetics of mercury compounds (e.g. Swensson & Uldvarson 1968, Scheuhammer 1988) and on their use as monitors of mercury pollution (review in Furness 1993, Monteiro & Furness 1995). It was shown that seabirds from remote areas exhibit some of the highest burdens of mercury without any apparent detrimental effects (Muirhead & Furness 1988, Thompson *et al.* 1993, Monteiro *et al.* 1995), although they were in the range that would cause toxic effects in waterfowl and terrestrial birds (Fimreite 1979, Nisbet 1994). Birds offer a variety of tissues as monitoring units (e.g. internal tissues, eggs, feathers, blood). But feathers, that are obtainable by non-destructive sampling, have gained increased popularity for monitoring, because of conservation, ethical and methodological reasons (Monteiro & Furness 1995, Chapter 7).

Among the vast avian mercury-related literature, which consists largely of baseline studies (review in Thompson 1990), emerged some detailed field studies that improved substantially the understanding of mercury dynamics in birds (e.g. Furness *et al.* 1986, Honda *et al.* 1986, Braune & Gaskin 1987, Thompson & Furness 1989a, Burger & Gochfeld 1992, Becker *et al.* 1993 and 1994, Stewart *et al.* 1994, Chapter 7). Experimental tracer studies with methylmercury and other mercury compounds are scanty. They have been undertaken mostly with poultry and always with captive birds (review in Fimreite 1979, March *et al.* 1983, Lewis & Furness 1991, 1993) and served to assess quantitatively toxicity, deposition and excretion rates, and dose-response relationships. Although such studies may provide in certain aspects a model of the typical wild bird, an experimental appraisal of methylmercury kinetics and dose-responses in free-living birds is still needed. In this respect, it is particularly relevant to fill the complete lack of

knowledge on the kinetics of methylmercury in avian blood, due to its role as internal carrier and ubiquous contact with all other tissues.

This study constitutes the first comprehensive evaluation of kinetics, dose-response relationships and excretion of methylmercury in free-living adult birds. A non-destructive multi-tissue approach, using blood, feathers and eggs was employed in a single-dose experimental design with the following objectives: (1) To determine the half-time of methylmercury in blood; (2) To derive relationships between steady-state dietary intake of methylmercury and blood concentrations; (3) To obtain partition coefficients for inter-tissue mercury concentrations; (4) To obtain relationships between mercury concentrations in parental blood and levels in eggs and hatchlings; (5) To obtain dose-response relationships between acute methylmercury dietary intake and levels in blood, eggs and hatchlings; (6) To quantify the deposition of mercury into eggs by females; (7) To assess mercury excretion through the skin and its adsorption into plumage; (8) To determine the relative contribution of distant and immediate dietary intake of mercury in the excretion into the plumage during the moult cycle. Furthermore, the potential toxicity of the doses administered, and assumed to be sub-toxic, was monitored and assessed.

9.2. METHODS

9.2.1. EXPERIMENTAL PROCEDURE

Cory's shearwater *Calonectris diomedea* was chosen as study-species because it is abundant, resistant to handling, has a long breeding season extending for eight months and exhibits strong nest site philopatry. Two experiments -A and B- were undertaken at a colony on Vila Islet, Azores during monthly visits of 8-10 days, between March-September 1994. There were three discrete dosing periods: late March (about a month after arrival), early May (three weeks before laying) and mid September (about a month before departure). Experiment A was designed in relation to objectives 1 to 7 and involved only the birds dosed in May. Experiment B was designed in relation to objective 8 and involved the birds dosed in March and September, as well some of the birds dosed in May.

Nest sites (burrows) were randomly assigned to different dose groups in each dosing period. In March and in September, 10 nest sites were assigned to

one of two dose groups of 1000μg or 2000μg. In May, 60 nest sites were assigned to one of three dose groups of 250μg, 1000μg or 2000μg. The species has nocturnal attendance at colonies and, on their return, adults were captured by hand at their burrows and were ringed, weighed, measured and, whenever possible, sexed (Granadeiro 1993). Subsequently, single-doses of methylmercury were administered orally in the form of analytical grade methyl mercuric chloride solution placed in gelatine capsules. After dosing, the birds were released into their burrow.

In experiment A, the birds dosed in May were recaptured to collect blood samples of 1 to 2 ml on days 2-5, 24-32, 51-61, 94-101 and 123-132 after dosing. Samples of growing (moulting or induced) ventral contour feathers (breast or belly) were collected along with blood samples, except on days 2-4. Blood samples from birds dosed in March with 1000 μg were also obtained on days 2, 46-47, 68-74, 98-108, 139-141 and 161-174 after dosing. Blood sampling was initiated on the second night after dosing to allow complete absorption of the ingested dose and to avoid collection of samples with high transient concentrations (Sherlock *et al.* 1984). Cory's shearwater lays a single egg in late May-early June. Eggs laid by females dosed in May were collected from 5-6 individuals by dose group within five days after laying, and fresh eggs abandoned by inexperienced breeders were provided as a 'replacement'. Down of all chicks hatched from eggs laid by females dosed in May was collected within two weeks of hatching. Blood, growing breast feather samples and abandoned eggs laid by non-dosed birds collected in the same periods served as controls (Chapter 7). On a return visit to Vila Islet in August 95, down of hatchlings from females dosed in May 1994 and from controls was collected.

In experiment B, during a return visit to Vila Islet in March 1995, a variety of contour feathers (breast, back, axillaries, scapulars, flank), primary one, primary ten and the outer rectrix were collected from recaptured birds, initially dosed in March, May and September 1994. Five birds were used as controls and sampled for the same tissues.

The doses were chosen to be within the range of exposures naturally experienced by the species, as estimated from four-fold increases between Atlantic and Mediterranean colonies (Renzoni *et al.* 1986). It was estimated that a dose of ~2800 μg was necessary to quadruple the plumage burden of mercury (5.4 μg/g * 65g; based on a elimination rate of 49% to the plumage, Lewis & Furness 1991). Although the dose range employed was assumed to be sub-toxic,

relevant biological information was collected to check for the most characteristic avian symptoms of mercury poisoning, which are, in ascending order of severity (Scheuhammer 1987): decreased egg hatchability, decreased egg production, reduced food intake leading to weight loss, impaired coordination.

9.2.2. Sample collection, preparation and mercury analysis

Sampling of plumage, blood and eggs, tissue preparation and storage, mercury analysis of tissues and analytical quality control were carried out as described in Chapter 4. All concentrations are given in microgram per gram on a fresh weight (ppm, fw) or dry weight (ppm, dw) basis.

9.2.3. Assumptions and data analysis

In this study, the analysis of mercury kinetics relies on two main assumptions. The first is that the single dose of methylmercury solution will be handled in the same way as doses absorbed repeatedly from the ingestion of food. This is partially supported by several tracer studies with humans that have shown an overall similarity in the kinetic parameters of methylmercury estimated from acute or chronic exposure via contaminated food (Kershaw *et al.* 1980, Sherlock *et al.* 1984). The second assumption is that concentration of total mercury in blood is a reliable indicator of changes due to the ingestion of methylmercury. Indeed, it was shown that concentrations of inorganic mercury did not change significantly in whole blood, plasma or red blood cells following single doses of methylmercury in humans (Kershaw *et al.* 1980). Furthermore, all mercury in blood of adult Cory's shearwater controls seems to be in the organic form (average organic:total mercury ratio = 107%, n = 3; this study), and this holds for plumage of a wide variety of seabirds (Thompson & Furness 1989b).

Total mercury concentrations determined in individual samples of tissues from experimental groups were corrected by subtracting the mean concentration found in the correspondent samples from controls. All subsequent analysis and calculations use these 'net' mercury concentrations, except where otherwise stated, and follow procedures outlined in Chapter 4. Due to difficulties in recapturing the same individuals at the various sampling periods, there were few complete individual time-series of blood samples. Hence, common estimates were used instead of individual estimates in kinetics data analysis. Values of the

variable time refer to days after complete absorption of the dose, thereafter time 0 = day 2 after ingestion.

9.2.4. PHARMACOKINETIC MODELS

9.2.4.1. Elimination of mercury in blood

The kinetics of methylmercury in whole blood of endotherm vertebrates is best known in humans and other primates. Hence, the mercury blood profiles with respect to time observed in Cory's shearwater were modelled accordingly. Single-dose studies with monkeys and humans revealed a rapid (30-90 min) absorption, an initial rapid decline, which appears to be complete about 20 to 30 h after the ingestion, followed by a much longer and slower decline (e.g. Kershaw *et al.* 1980, Rice *et al.* 1993). The concentration of mercury $C(t)$ (µg/g) at time t (days) in the blood following complete absorption of a single oral dose is given by a two compartment model, corresponding to the initial rapid phase and the slower terminal phase (Rice *et al.* 1993, Sällsten *et al.* 1993),

$$C(t) = A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t} \qquad (9.1)$$

where A, B (µg/g) and α, β (1/day) are unknown parameters to be estimated on the basis of experimental data. A one compartment model may be used to describe solely the terminal elimination, which is the relevant phase in the assessment of equilibrium concentrations (Kershaw *et al.* 1980, Sherlock *et al.* 1984),

$$C(t) = A \cdot e^{-\alpha \cdot t} \qquad (9.2)$$

$C(t)$ is proportional to $e^{-\delta \cdot t}$, where $\delta = \max \{\alpha, \beta\}$ for the initial phase and $\delta = \min \{\alpha, \beta\}$ for the terminal phase in (9.1), and $\delta = \alpha$ in (9.2). In each phase, the half-time ($T_{½}$, days) is given by

$$T_{½} = (\ln 2) / \delta \qquad (9.3)$$

9.2.4.2. Intake and equilibrium blood concentrations

Dose-response models for mercury at equilibrium in whole blood of adult Cory's shearwater were approached using the following equations in use for humans and other primates.

The relationship between the steady state concentration of methylmercury in blood (C, µg/kg) and the daily intake (I, µg/day) is identical in single-dose and chronic dosing studies (Sherlock *et al.* 1984) and is given by (Kershaw *et al.* 1980)

$$C = I \,.\, (f \,/\, V) \,/\, \delta \qquad (9.4)$$

where f is the fraction of the daily intake deposited in blood, V is the volume of blood (ml, i.e. g) and $\delta = (\ln 2) \,/\, T_{½}$ is estimated from models (9.1) or (9.2). The parameter f is given by

$$f = V \,.\, A \,/\, D \qquad (9.5)$$

where A, the intercept of the slow component estimated from models (9.1) or (9.2), represents the blood concentration that would have resulted if absorption and tissue distribution were complete at time zero (Kershaw *et al.* 1980), and D (µg) is the dose.

Assuming the blood volume is 7% of the body mass as in birds in general (W, g; Sturkie 1986),

$$C = I \,.\, (f \,.\, T_{½}) \,/\, (0.07 \,.\, \ln 2 \,.\, W) = I \,.\, a \qquad (9.6)$$

where a (days/g) represents the number of days intake of methylmercury contained in 1 g of circulating blood and is given by

$$a = (f \,/\, 0.07) \,.\, (T_{½} \,/\, \ln 2 \,.\, W) \qquad (9.7)$$

9.3. RESULTS

9.3.1. General

Cory's shearwater is dimorphic in size with males being larger than females (Subchapter 2.2) and, hence, the doses administered resulted in a larger load of methylmercury in females compared to males. The doses normalised to body mass (D_n, µg/g) averaged, respectively, in males and females, for the 250µg, 1000µg and 2000µg dose groups: 0.27 and 0.33 (pooled sexes 0.30), 1.11 and 1.29 (pooled sexes 1.22), 2.29 and 2.64 (pooled sexes 2.47). On average, females were subjected to mercury loads 16-20% higher than males.

Differences between days in average blood mercury concentrations within the initial period (days 0 to 3) approached significance especially in the two higher dose groups (Table 9.1). Despite small sample sizes, there is a tendency for decreasing levels across that period, which supports the assumption that absorption was completed at time zero.

TABLE 9.1. - Comparison of mercury concentrations (µg/g dw; mean ±S.E., sample size bracketed), measured at various days (time 0 = 2 days after dosing) within the initial sampling period, for each dose group.

Time	Dose (µg)		
(days)	250	1000	2000
0	3.1 ±0.2 (5)	13.0 ±0.4 (21)	25.8 ±0.9 (15)
1	2.6 ±0.2 (9)	10.3 ±2.5 (2)	22.0 ±1.9 (5)
2	3.0 ±0.7 (5)	–	–
3	2.4 ±0.4 (2)	–	–
	$F_{3,17} = 2.34$* P = 0.11	$t_{21} = 2.02$** P = 0.056	$t_{18} = 2.07$** P = 0.053

* 1-way ANOVA, ** t-test

9.3.2. KINETICS OF METHYLMERCURY IN BLOOD

9.3.2.1. Elimination

The mean concentrations of mercury in blood of adult Cory's shearwater collected at various time periods after dosing in May declined in a highly significant way without significant effect of sex for each dose group (Table 9.2). Thereafter, data from pooled sexes will be used in kinetic analysis. Detectable levels of methylmercury remain in the blood at over 90 days for the group dosed with 250µg and at over 120 days for the groups dosed with 1000µg and 2000µg.

The blood methylmercury concentration profiles with respect to time are better fitted by the two compartment models than by the one compartment model. The estimates of the parameters in model (9.1) fitted to data from the three dose groups from May are given in Table 9.3 and Figure 9.1 illustrates the fit of the model for the 1000µg dose group. The estimated half-times of methylmercury in blood during the terminal elimination phase varied between 40 and 46 days among dose groups, for birds dosed in May (Table 9.3). A comparable longer half-time of 65 days was obtained from a set of birds dosed with 1000µg in March and followed up for about 170 days. Although the data did not allow a confident assertion of the half-time of the initial rapid phase, especially for the lower dose

TABLE 9.2. - Comparison of blood mercury concentrations (µg/g dw; mean ±S.E., sample size bracketed), measured at various times (mean, range bracketed) after dose administration in early May, for each dose by sex.

Time	Dose 250 µg		Dose 1000 µg		Dose 2000 µg	
(days)	Male	Female	Male	Female	Male	Female
0.5 (0-2)	2.9 ±0.3 (12)	2.6 ±0.1 (9)	12.5 ±0.5 (13)	13.2 ±0.6 (10)	24.4 ±1.2 (11)	25.5 ±1.3 (9)
28.2 (24-32)	2.4 ±0.4 (11)	1.4 ±0.3 (10)	5.5 ±0.2 (11)	5.3 ±0.4 (11)	10.6 ±0.5 (12)	10.4 ±0.6 (10)
56.3 (51-61)	1.4 ±0.3 (11)	0.8 ±0.2 (11)	3.6 ±0.4 (10)	3.8 ±0.3 (12)	8.5 ±0.5 (11)	8.4 ±0.5 (11)
97.1 (94-101)	0.2 ±0.1 (7)	0.7 ±0.3 (6)	1.5 ±0.3 (6)	1.7 ±0.2 (7)	2.9 ±0.2 (7)	3.5 ±0.4 (6)
128.5 (123-132)	0.0 ±0.1 (6)	0.2 ±0.2 (4)	1.1 ±0.1 (6)	0.7 ±0.2 (8)	1.3 ±0.2 (6)	1.6 ±0.2 (4)
2-way ANOVA, Effect:						
Month	$F_{4,76}=21.94$, $P<0.0001$		$F_{4,84}=287.12$, $P<0.0001$		$F_{4,77}=268.90$, $P<0.0001$	
Sex	$F_{1,75}=1.21$, $P=0.27$		$F_{1,84}=0.18$, $P=0.67$		$F_{1,77}=0.47$, $P=0.49$	

TABLE 9.3. - Correlation, parameter estimates and half-times derived from kinetic bivariate and multi-dose two compartment models fitted to data of adult Cory's shearwater dosed in May.

Model	Dose	r	A	α	B	β	$T_{\frac{1}{2}}$ (days)	
(equation)	(μg)		(μg/g)	(1/days)	(μg/g)	(1/days)	initial phase	terminal phase
Bivariate	250	0.70	1.53	0.0173	1.39	0.0173	–	40
(9.1)	1000	0.97	8.62	0.0165	4.38	0.855	0.8	42
	2000	0.96	17.0	0.0150	8.87	0.507	1.4	46
Multi-dose	pooled	0.97	–	0.0157	–	0.816	0.8	44
(9.8)	250		2.14		1.10			
	1000		8.56		4.39			
	2000		17.82		9.04			

Fig. 9.1
Decline of mercury in blood of Cory's shearwater dosed orally with 1000 ug of methylmercury in May.

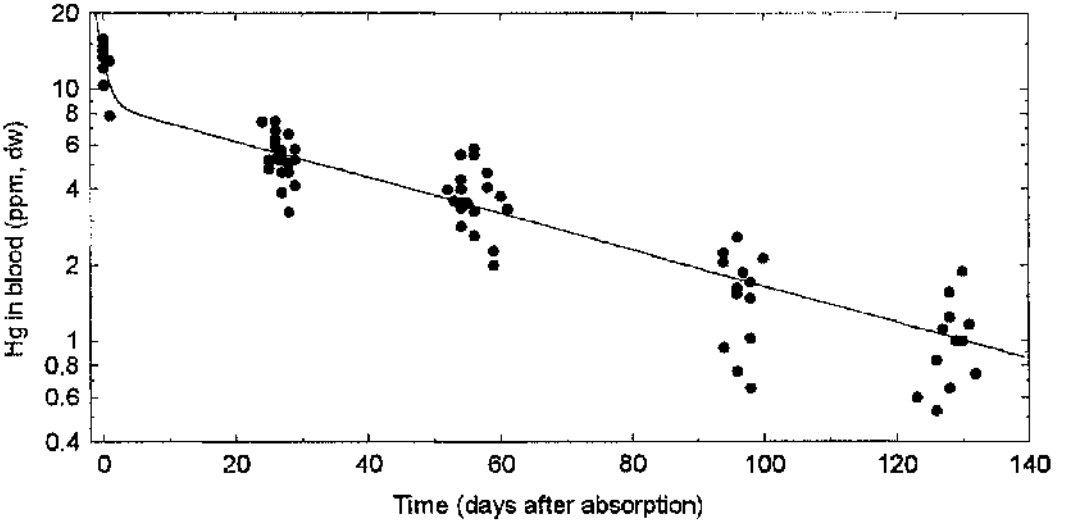
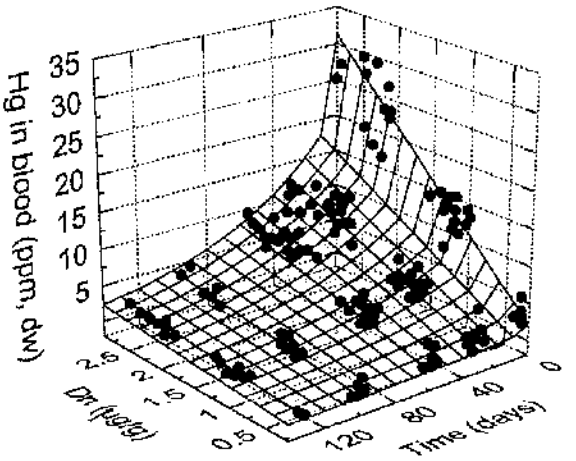


Fig. 9.2
Multi-dose model for decline of mercury in blood of adult Cory's shearwater dosed orally with methylmercury in May.



group, an order of magnitude around 1 day is inferred from the highest values of β in Table 9.3. The poor fit of data from the lower dose group might account for the odd estimate of β for that group.

Given that the dose-response of methylmercury in blood is linear (see section 9.3.4.1), a multi-dose version of model (9.1), derived by incorporating the dose normalised to body mass (D_n, µg/g),

$$C(t) = D_n \,.\, (A \,.\, e^{-\alpha \,.\, t} + B \,.\, e^{-\beta \,.\, t}) \qquad (9.8)$$

was fitted to the pooled-dose elimination profiles of methylmercury in blood of birds dosed in May (Fig. 9.2; r = 0.97, n = 268):

$$C = Dn \,.\, (7.13 \,.\, e^{-0.0157.t} + 3.66 \,.\, e^{-0.816.t}) \qquad (9.9)$$

This model fits the experimental data well and produces overall estimates of parameters and half-times in the initial and terminal phase identical to those of the bivariate model.

9.3.2.2. Intake and equilibrium blood concentrations

Using the intercepts of the slow component obtained from the bivariate and multi-dose elimination models (A, Table 9.3), a dose-response relationship for equilibrium methylmercury concentrations in blood of adult Cory's shearwater was derived using equations (9.4) through (9.7) in the methods section. An average blood volume of 60 ml (i.e. 12g on a dry weight basis) was assumed. Estimates

TABLE 9.4. - Estimated fraction of dose deposited in the blood volume (*f*) for each dose based on equation (9.5). Values of *A* were intercepts of the slow component obtained from the bivariate (9.1) and the multi-dose (9.8) models.

Dose	*f* based on *A* derived from equation	
(µg)	(9.1)	(9.8)
250	0.0734	0.103
1000	0.103	0.103
2000	0.102	0.107

of the fraction of dose deposited in the blood volume (*f*) varied between 7.3 and 10.7% (Table 9.4). However, estimates of *f* derived using the intercepts from the multi-dose model were more consistent, ranging between 10.3 and 10.7% among the three dose groups (Table 9.4). Therefore, an overall *f* of 0.104 and half-time of 44 days were employed in equation (9.7) to derive the relationship between steady state methylmercury concentration in blood (*C*, µg/g fw) and daily intake (*I*, µg/day) for a given body mass (*W*, g)

$$C = I \cdot (94.3 / W) \qquad (9.10)$$

9.3.3. INTER-TISSUE RELATIONSHIPS

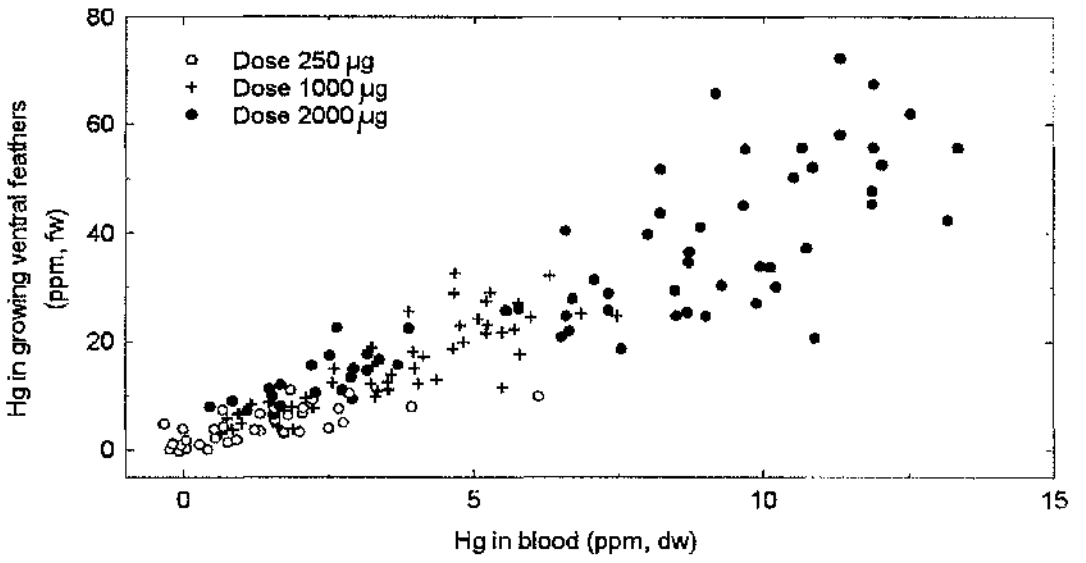
9.3.3.1. Blood:growing ventral feathers

The relationships between mercury concentrations in blood and growing ventral feathers collected simultaneously from individual Cory's shearwater from June to September are best described by a linear model and did not differ significantly between sexes for each dose group (ANCOVAs): 250µg, $F_{1,62}=2.44$, $P=0.12$; 1000µg, $F_{1,68}=6.03E\text{-}4$, $P=0.98$; 2000µg, $F_{1,64}=5.57E\text{-}3$, $P=0.94$.

The scatterplot of mercury concentrations in growing ventral feathers (VF) and blood (BL) is shown in Fig. 9.3 by dose group. The regression lines (constrained to pass through the origin) for each group were significantly different (ANCOVA, $F_{2,199}=4.67$, $P<0.05$):

Fig. 9.3

Scatterplot of mercury concentrations in growing ventral feathers and blood.



250µg: $r = 0.88$, $F_{1,64} = 222.03$, $P < 0.0001$; VF = 2.9 ± 0.2 S.E. * BL (9.11)

1000µg: $r = 0.97$, $F_{1,70} = 1124.2$, $P < 0.0001$; VF = 4.1 ± 0.1 S.E. * BL (9.12)

2000µg: $r = 0.97$, $F_{1,66} = 904.6$, $P < 0.0001$; VF = 4.3 ± 0.1 S.E. * BL (9.13)

9.3.3.2. Parental blood:egg

To estimate concentrations at the start of the lag period with deposition of albumen (Grau 1984), i.e. the main mercury store in the egg (Chapter 7), mercury concentrations in blood of females sampled within five days of laying were increased by a factor of 16% to correspond to the average decline in blood levels during a ten days period. An highly significant linear relationship ($r = 0.98$, $F_{1,14} = 454.38$, $P < 0.0001$) was observed between such corrected blood concentrations (BL) and those in eggs (EG) they laid (Fig. 9.4):

$$EG = 0.94 \pm 0.04 \text{ S.E. } * BL \quad (9.14)$$

9.3.3.3. Parental blood:down of hatchling

Mercury concentrations in blood (BL) of females sampled within five days after laying (corrected as above) and concentrations in down (D1) of their chicks within two weeks of hatching showed a highly significant linear relationship ($r = 0.93$, $F_{1,8} = 52.40$, $P < 0.0001$; Fig. 9.5):

$$D1 = 2.6 \pm 0.4 \text{ S.E. } * BL \quad (9.15)$$

Fig. 9.4
Relationship between mercury concentrations in blood of females and in their eggs. Fitted line constrained to pass through the origin.

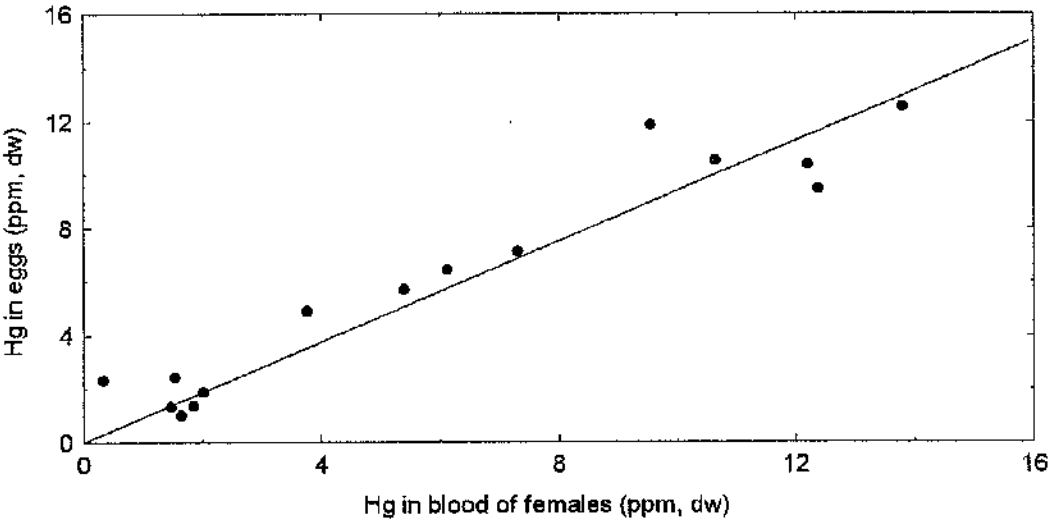
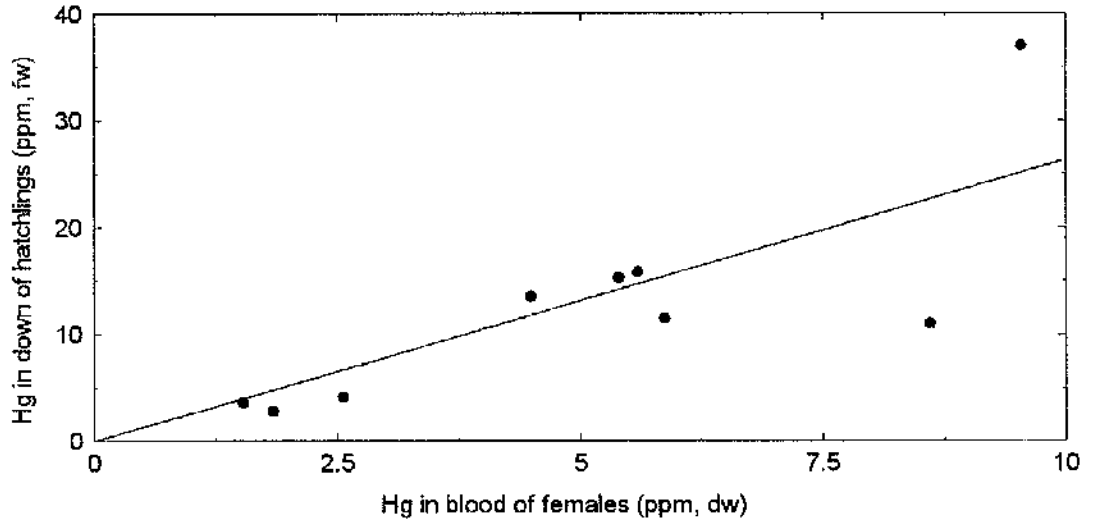


Fig. 9.5
Relationship between mercury concentrations in blood of females and in down of their hatchlings. Fitted line constrained to pass through the origin.

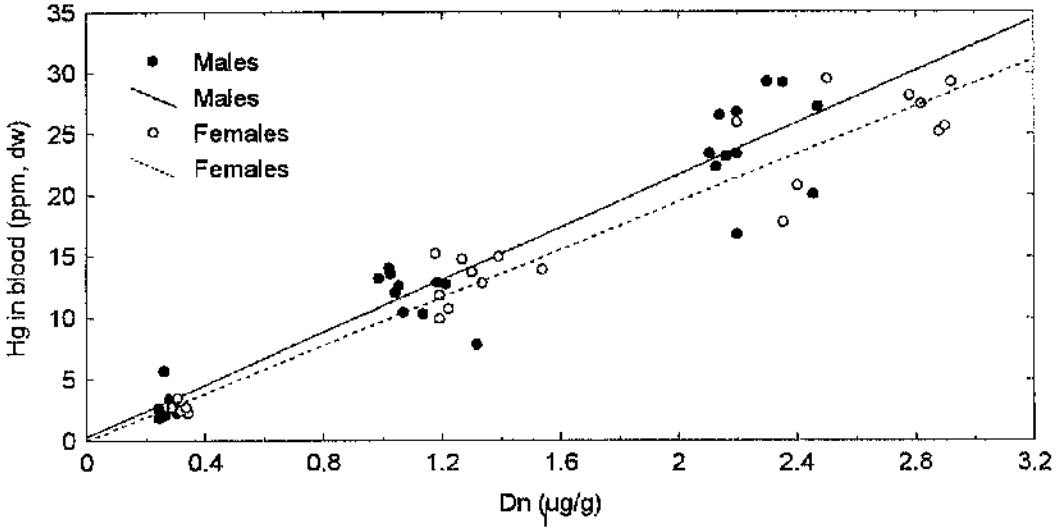


9.3.3.4. Egg:down of hatchling

Though a direct relationship between mercury concentrations in the egg and down of hatchling cannot be obtained because measuring mercury in eggs is destructive, it may be derived by combination of equations 9.14 and 9.15. The resulting egg (EG, µg/g dw)-down (D1, µg/g fw) relationship is

$$D1 = 2.8 * EG \qquad (9.16)$$

Fig. 9.6
Dose-response relationship for blood mercury concentration immediately after exposure, for each sex. Fitted lines constrained to pass trough the origin.



9.3.4. DOSE-RESPONSE RELATIONSHIPS

9.3.4.1. Blood of adults

Blood mercury concentrations in the initial period after dosing (time 0 to 2 days) increased linearly and significantly with dose normalised for body mass (D_n), both for males ($r=0.99$, $F_{1,33}=1182.9$, $P<0.0001$) and females ($r=0.98$, $F_{1,25}=1439.5$, $P<0.0001$; Fig. 9.6):

$$\text{Males:} \quad Hg = 10.84 \pm 0.32 \text{ S.E. } * Dn \qquad (9.17)$$

$$\text{Females:} \quad Hg = 9.72 \pm 0.26 \text{ S.E. } * Dn \qquad (9.18)$$

Females exhibited a slope 10% smaller and significantly different from that of males (ANCOVA, $F_{1,57}=5.40$, $P<0.05$), although they were subjected to relative mercury loads higher than males by over 16%.

9.3.4.2. Eggs

Mercury concentrations in the whole egg contents show a highly significant positive linear relationship with dose normalised to female body mass (D_n) ($r=0.99$, $F_{1,15}=1018.3$, $P<0.0001$; Fig. 9.7):

$$Hg = 4.2 \pm 0.1 \text{ S.E. } * Dn \qquad (9.19)$$

Fig. 9.7
Relationship between dose and mercury concentrations in eggs.
Fitted line constrained to pass through the origin.

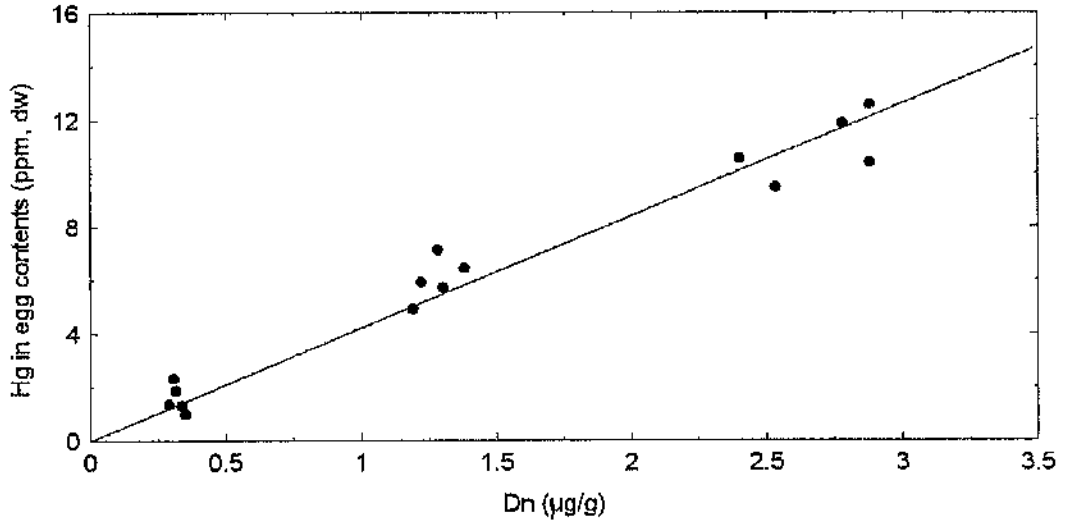
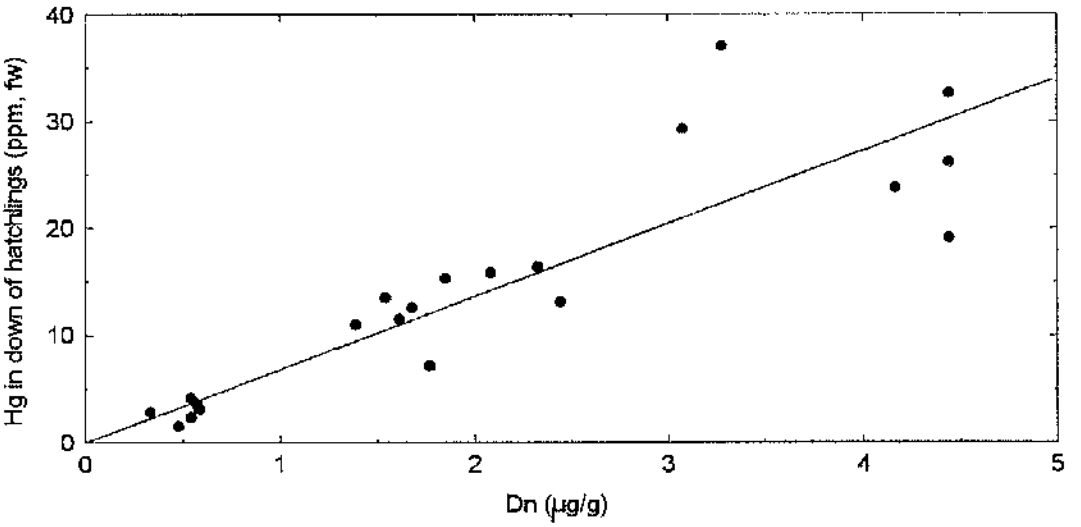


Fig. 9.8
Relationship between dose and mercury concentrations in down of hatchlings.
Fitted line constrained to pass through the origin.



9.3.4.3. Down of hatchlings

Mercury concentrations in down of hatchlings show a highly significant positive linear relationship with dose normalised to females body mass (D_n) ($r = 0.99$, $F_{1,21} = 249.9$, $P < 0.0001$; Fig. 9.8):

$$Hg = 6.8 \pm 0.4 \text{ S.E.} * Dn \qquad (9.20)$$

TABLE 9.5. - Mercury excretion in eggs laid by adults dosed in May. Values are mean ± S.E.

Dose (µg)	n	Total Hg excreted (µg)	Total Hg excretion as % of intake
250	6	37 ±4	15 ±2
1000	6	134 ±8	13 ±1
2000	5	238 ±11	12 ±1

9.3.5. EXCRETION

9.3.5.1. Eggs

Table 9.5 shows amounts and percentages of immediate mercury excretion into the whole egg contents for the three dose groups from May, since mercury does not accumulate in the shell (Burger *et al.* 1994, Chapter 7). The level of the dose administered had no significant effect on the percentage excreted (1-way ANOVA, $F_{2,14} = 1.74$, $P = 0.21$), which averaged 14% of the intake. Mercury excretion into the albumen component ranged between 89 to 94% of the total, in a sample of 6 eggs from different dose groups (1 of 250µg, 3 of 1000µg and 2 of 2000µg). This compares with an average of 89% observed in controls (Chapter 7).

Long-term excretion of the 'pulse' of methylmercury into eggs laid in the following breeding season (1995) appears to be nill, as indicated by absolute mercury concentrations (not corrected for controls) in down of hatchlings from eggs laid by control females (5.0 ±0.3, n = 10) and those dosed in May 1994 with 250µg (4.9 ±0.7, n = 5), 1000µg (5.2 ±0.4, n = 5) and 2000µg (5.0 ±0.4, n = 5), which do not differ significantly (1-way ANOVA, $F_{3,22} = 0.098$, $P = 0.96$).

9.3.5.2. Skin

Mercury concentrations in breast feathers formed prior to the exposure to mercury, were compared between samples collected from the same individuals immediately before dosing (May) and one month later (June) to assess short-term excretion of dietary mercury into plumage by adsorption of contaminated exfoliated epidermal cells (March *et al.* 1983). Mean mercury concentrations (not corrected for controls) increased significantly between pre- and post-exposure

TABLE 9.6. - Change in mercury concentrations (not corrected for controls; μg/g fw; mean ±S.E.) of fully grown breast feathers collected immediately before dosing (May) and one month later (June), by dose group.

Month	Dose (μg)		
	250	1000	2000
May	5.7 ±0.3	5.5 ±0.4	6.2 ±0.4
June	6.3 ±0.4	6.3 ±0.4	8.8 ±0.6
	$t_8 = 2.53$* P<0.05	$t_9 = 4.04$* P<0.005	$t_9 = 5.92$* P<0.0005
Change			
(μg/g)	+0.6	+0.8	+2.6
(%)	10.5	14.5	41.9

* paired t-test

samples in all dose groups (Table 9.6). Conservative crude estimates of the percentage of the intake eliminated trough the skin, derived by multiplying the mean increase in breast feather concentration by a mass of contour plumage of 25g (Monteiro unpubl. data), range from 2.6 to 6.0% among the three dose groups.

9.3.5.3. Plumage

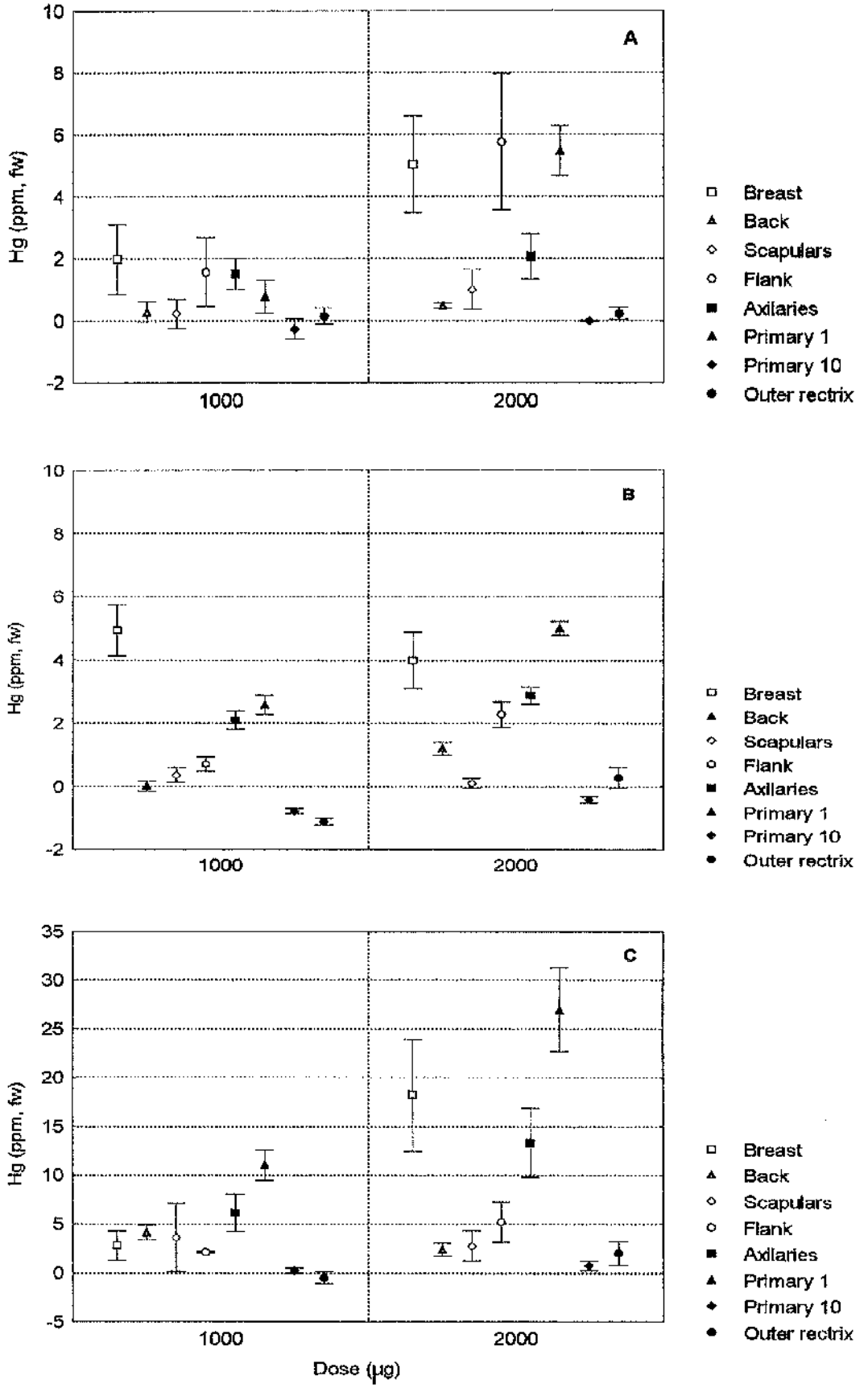
The excretion of dietary mercury into plumage by endogenous incorporation during feather formation was assessed by determining mercury concentrations in different feather types of adult Cory's shearwater dosed at three different occasions of the breeding season (March, May, September) on recapture, in March of the following year, after a complete moult cycle (Table 9.7, Fig. 9.9). Significant responses to the 'pulse' of mercury were obtained only in certain feather types and these responses were stronger in birds dosed in September. Endogenous excretion of the intake into the plumage was estimated by using a mean plumage mercury concentration, resulting from the arithmetic mean of concentrations in the eight feather types, multiplied by a mean plumage mass of

TABLE 9.7. - Mercury concentrations (µg/g fw; mean ±S.E.) in different feather types of adult Cory's shearwater dosed in March, May or September and recaptured in March of the following year, after a complete moult cycle. Mean values in bold and italic differ signifficantly from a mean of zero (t-tests, $P<0.05$). Estimates of the total excretion into plumage are given at the bottom of table. For explanation of the calculation basis of excretion see text.

Feather type	March		May		September	
Dose (n)	1000 µg (6)	2000 µg (3)	1000 µg (5)	2000 µg (5)	1000 µg (3)	2000 µg (5)
Breast	***2.0 ±1.1***	***5.0 ±1.6***	***4.9 ±1.2***	***4.0 ±1.3***	***2.8 ±1.5***	***18.2 ±5.7***
Back	0.3 ±0.3	***0.5 ±0.1***	0.0 ±0.2	***1.2 ±0.3***	***4.2 ±0.8***	***2.4 ±0.7***
Scapulars	0.2 ±0.4	1.0 ±0.6	0.4 ±0.4	0.1 ±0.2	3.7 ±3.4	2.8 ±1.5
Flank	1.6 ±1.1	5.8 ±2.2	0.7 ±0.3	2.3 ±0.6	***2.1 ±0.1***	***5.2 ±2.2***
Axilaries	***1.5 ±0.4***	***2.1 ±0.7***	***2.1 ±0.4***	***2.9 ±0.4***	***6.2 ±2.3***	***13.3 ±3.5***
Primary 1	0.8 ±0.5	***5.5 ±0.8***	***2.6 ±0.4***	***5.0 ±0.3***	***11.1 ±1.6***	***27.0 ±4.3***
Primary 10	-0.3 ±0.3	0.0 ±0.0	***-0.7 ±0.1***	***-0.4 ±0.2***	0.3 ±0.3	0.8 ±0.4
Outer rectrix	0.2 ±0.3	0.2 ±0.2	***-1.1 ±0.2***	0.3 ±0.5	-0.4 ±0.6	2.1 ±1.2
Mean concentration in plumage	0.8	2.5	1.1	1.9	3.8	9
Total excretion into plumage (µg)	52	163	72	124	247	585
Excretion as % of intake	5.2	8.2	7.2	6.2	24.7	29.3

Fig. 9.9

Mercury concentrations (mean ±SE) in the plumage of adults dosed in March (A), May (B) and September (C), recaptured next March at the end of the moult cycle.



65g (Monteiro unpubl. data). There is a good agreement between rates of elimination in the two dose groups for each dosing period. The overall percentage of elimination is identical for birds dosed in March and May (7%), which is lower that of birds dosed in September (27%).

9.3.6. Toxicity

Potential dose-induced toxicity was assessed by comparison among dose groups and controls of: (1) egg production and hatching success; (2) hatchling and pre-fledgling body condition (as indicated by body mass); (3) chick growth; (4) adult body condition (as indicated by body mass).

Egg production, defined as nests with egg laid out of nests with dosed pair, was relatively uniform among the three dose groups: 250µg -88%, n=16; 1000µg -95%, n=20; 2000µg -79%, n=19. Hatching success of eggs laid and incubated by dosed birds was uniform among the three dose groups (250µg -75%, n=8; 1000µg -79%, n=14; 2000µg -67%, n=9) and identical to that of eggs in control nest sites (77%, n=94; Sub-chapter 2.2). Overall hatching success of 'replacement' eggs (see Methods) was 44% (n=16).

There was no significant effect of dose (controls included) on chick body mass measured within two weeks of hatching (ANCOVA, $F_{3,43}=0.48$, $P=0.70$; wing length as covariate) nor within two weeks before fledging (ANCOVA, $F_{3,24}=0.72$, $P=0.55$; wing length as covariate). Moreover, parameters of growth curves in the form of a quadratic function of body mass on wing length, fitted to data from individual chicks, did not vary significantly among dose groups and controls (Table 9.8).

There was no significant effect of dose on adult body mass between dosing in May and September 1994 (ANCOVA, $F_{3,428}=1.77$, $P=0.15$; sex and month as covariates; all three dose groups and controls included) and in March 1995 (ANCOVA, $F_{2,26}=1.63$, $P=0.22$; sex as covariate; 1000µg and 2000µg dose groups and controls included).

TABLE 9.8. - Parameters for the quadratic function of body mass (BM) on wing length (WL) fitted to growth curves of individual chicks from adults of the three dose groups of May and controls. Values are mean ±S.E.

Dose (µg)	n	Parameters for growth curve $BM = A + B*WL + C*WL^2$		
		A	B	C x100
250	6	166 ±53	7.1 ±0.6	-1.3 ±0.2
1000	7	128 ±57	7.4 ±0.9	-1.4 ±0.2
2000	6	114 ±82	8.1 ±1.1	-1.5 ±0.3
0	8	214 ±43	6.6 ±0.7	-1.2 ±0.2
1-way ANOVA		$F_{3,23}=0.63$, P=0.60	$F_{3,23}=0.52$, P=0.67	$F_{3,23}=0.29$, P=0.83

9.4. DISCUSSION

9.4.1. KINETICS OF METHYLMERCURY IN BLOOD

9.4.1.1. Validity aspects

Mammalian tracer studies have shown a quick absorption of ingested methylmercury (<10 h) and an initial rapid decline, completed within 30 h after ingestion (Kershaw *et al.* 1980, Sherlock *et al.* 1984, Rice *et al.* 1989). That served to design the sampling periodicity of blood to follow the elimination of methylmercury during the terminal phase, the only phase relevant in the assessment of equilibrium concentrations (Kershaw *et al.* 1980, Sherlock *et al.* 1984). Later in the season, a pilot-assessment suggested a much slower absorption phase in adult-sized Cory's shearwater chicks, with peak blood concentrations being attained at 24 h after single-dose administration and being sustained until 48 h after ingestion (Chapter 10: Fig. 10.1). A similar period is anticipated for Cory's shearwater adults, since the absorption kinetics is thought to be governed essentially by the volume of the body pool. This is supported from data in Table 9.1, due to lack of high transient concentrations on days 0 to 3, i.e. days 2 to 5 after ingestion. It implies that the time blood profiles obtained in this study include the initial elimination phase, and hence were best fitted by the two

compartment model. Unfortunately, the poor coverage of the initial phase, not anticipated in the experimental design, rendered impossible an accurate assessment of the respective parameters, especially the intercept of the fast phase. The parameter estimates for the slow phase, however, should not be affected.

9.4.1.2. Elimination

Estimates of blood methylmercury half-times in the initial elimination phase varied between 0.8 and 1.4 days for the higher dose groups. Comparative half-times in humans average 0.3 days (Kershaw *et al.* 1980). This suggests a faster equilibrium between the blood compartment and extra-vascular tissues in humans than in Cory's shearwater. Blood methylmercury half-times in the terminal elimination phase ranged from 40 to 46 days, for birds dosed in May, and an estimate of 65 days was obtained from the blood profiles of birds dosed in March with 1000 μg. Such a difference in half-times seems to derive from a moult effect. Moult of breast and other contour feathers initiates in June (Chapter 6) and, hence, birds dosed in May would have opportunity to excrete the 'pulse' of methylmercury into growing feathers faster than birds dosed in March. Therefore, moult emerges as a crucial factor in determining blood methylmercury half-times in birds. Comparative avian blood half-times are not available in the literature, but whole-body half-times are in the order of 70 to 85 days, with moult-related uneven variations (review in Fimreite 1979). Half-retention times in tissues of experimentally exposed chickens decreased markedly with increasing exposure level and, for the lower groups ranged between 14 days in liver to 59 days in muscle (March *et al.* 1983). Comparative mammalian blood half-times averaged 15 to 50 days in macaques and monkeys (Rice *et al.* 1993) and 50 to 52 days in humans (Sherlock *et al.* 1984). In this study, the half-times in Cory's shearwater increased from 40 to 46 days in relation to a eight-fold increase in the dose (dose range: 0.3-2.5 μg/g). This compares with an increase in humans from 46 to 53 days in relation to a five-fold increase in dose (dose range: 0.06-0.3 μg/g; Sherlock *et al.* 1984). Significant sexual differences are not evident in blood mercury time-profiles of Cory's shearwater (cf. Table 9.2). However, sex-related differences in kinetics, namely half-times, arising from laying and presumed variations in moult chronology (Chapter 6), are predictable but their assessment would require a special experimental design.

There is no elaborate or validated metabolic model for avian exposure to methylmercury. The blood half-time estimates presented in this study were an essential basis for a future quantitative compartment model. The initial fast phase probably reflects the distribution of methylmercury in one compartment for which transport is characterised as flow limited (i.e. liver, kidneys and other target organs). The second phase may reflect loss to a second compartment for which transport is diffusion limited (i.e. faeces, the intestinal lumen and plumage).

9.4.1.3. Intake-equilibrium blood concentrations

The estimate of the average fraction of ingested methylmercury deposited in the blood volume of Cory's shearwater (average 0.104) compares with estimates in humans averaging 0.056 and 0.059 (Kershaw *et al.* 1980, Sherlock *et al.* 1984). The higher deposition of methylmercury in blood of Cory's shearwater than that of humans might result from one or a combination of the following factors: (1) mercury intake higher in order of magnitude than that of humans; (2) inter-species differences in the ratio between methylmercury in red blood cells and plasma (approx. 95% of the human blood mercury is in the erythrocytes, WHO 1990); (3) inter-species differences in methylmercury behaviour in erythrocytes (Naganuma *et al.* 1980). The current estimate of the fraction of ingested methylmercury deposited in blood of Cory's shearwater is thought to be representative of that in repeated ingestion under natural conditions, judging from the similarity of the estimates obtained in single-dose (Kershaw *et al.* 1980) and repeated-dosing (Sherlock *et al.* 1984) studies with humans.

The knowledge of methylmercury terminal half-time in blood and of the fractional deposition in blood of the ingested dose, allowed a first insight into the relationship between avian steady-state blood concentrations and dietary intake of methylmercury. Moreover, a combination of this relationship with other tissue-blood relationships would allow direct tissue concentration-dietary intake relationships for feathers, eggs and down of hatchlings. These sort of relationships are thought to be of general application to other bird species over a wide range of intakes, since results from this study show little evidence of dose-dependent kinetics (half-times, fractional deposition in blood) over a wide dose range. However, the effect of moult on avian blood half-times represents an uncertainty to be considered when employing such relationships.

9.4.2. Partition coefficients

The slopes of the tissue-blood relationships obtained in this study represent the best avian estimate of the levels of mercury which partitioned into the tissues. As all those tissues have vestigial levels of inorganic mercury, such slopes may well be representing methylmercury avian partition coefficients. There is no evidence of major dose-dependency in the current partition coefficients judging from the linearity of the relationships, despite feather-blood partition coefficient were slightly higher in the higher dose groups. This may be accounted for by highly transient declining blood concentrations in these groups, so that feather concentrations reflect current but also previous blood concentrations. Overall, the partition coefficients obtained do not differ more than 25% from those observed in controls (Chapter 7) and were thought to be adequate to use in advanced modelling of the kinetics of methylmercury in birds. The avian feather-blood partition coefficients estimated in this study (2.9-4.3) are a order of magnitude lower than the value of 50 for simultaneous hair-blood concentrations (both in ppm, dw) in humans (WHO 1990).

9.4.3. Dose-responses

All dose-response relationships determined in this study were linear over the wide range of exposures employed. This results agreed with other avian studies that showed linear dose-responses for methylmercury in eggs of domestic fowl (Tejning 1967), feathers and tissues of Black-headed gull *Larus ridibundus* chicks exposed to low doses (Lewis & Furness 1991) and tissues of Zebra finches *Poephila guttata* chronically exposed over a wide dose range (Scheuhammer 1988). Immediate dose-responses in avian eggs (e.g. Lewis & Furness 1993, this study) validate the assumption that their mercury concentrations represent dietary exposure shortly before their formation (Furness 1993).

The sex-related difference in the blood dose-response observed in this study is noteworthy. Indeed, females were subjected to mercury loads 16-20% higher than males and exhibit a lower dose-response by about 10%. Since dosing took place three to four weeks before laying, and deposition of albumen in large seabirds (the main egg-mercury store) initiates about a week before laying (Grau 1984), a lower dose-response in females arising from direct deposition into the egg seems to be ruled out. Potential, but unidentified, sex-related differences in physiology may be a cause for inter-sex differences in mercury dynamics.

Dose-response linearity under natural exposure ranges (Lewis & Furness 1991, this study) provides a strong validation of the assumption that no saturation of the metal-accumulatory ability is operating in seabird tissues currently used as monitoring units. That enables the direct assessment of variations in exposure through diets (and potential variations in extent of contamination on the assumption of constant diets) from concentrations in seabird tissues. For instance, mercury concentrations in eggs of Cory's shearwater (Renzoni *et al.* 1986, Chapter 7) indicate an increase up to four times in dietary intake of mercury from the Atlantic to the Mediterranean.

9.4.4. Excretion

The major avian eliminatory pathways for mercury are the plumage, faeces and urine, and, in females, the eggs (e.g. Braune & Gaskin 1987, Monteiro & Furness 1995). In this study, female Cory's shearwater excreted into the single egg an average of 14% of the dose ingested three to four weeks before laying, without evidence of dose-dependency excretion. This rate compares with 30% of a 120 μg dose excreted by quail *Coturnix coturnix* (which lays approx. 1 egg/day) in eggs laid within one week after administration (Lewis & Furness 1993). A mercury budget for Herring gull *Larus argentatus* also indicate that female may excrete over 20% more mercury via their eggs than could be excreted by males (Lewis *et al.* 1993).

Mercury excretion into the plumage attained a higher rate (approx. 27% of the intake) in the group dosed three months after start of the moult cycle (September), compared to the overall 7% excreted in the groups dosed two and one month before the start of the moult cycle (March and May, respectively) (cf. Chapter 6). Although sample sizes were small, there is no evidence of dose-dependency on percentage excretion, likewise in feathers and tissues of gull chicks exposed to low doses of methylmercury (Lewis & Furness 1991). In contrast, exposure to higher doses in chickens resulted in dose-dependent excretion in internal tissues. The excretion rates presented here did not account for mercury elimination into feather sheaths (Burger *et al.* 1992) and were lower than values found in the literature. Half or more of the avian body burden may be removed into the plumage by chicks growing an entire plumage (49%, Lewis & Furness 1991) and by adults during a moult cycle (59-68%, Braune & Gaskin 1987). Indeed, the highest excretion rate obtained in this study is about half of

those in literature and this may simply derive from the fact that dose administration was undertaken with new feathers already grown at time of dosing. It is noteworthy that doses administered one to two months before start of the moult cycle produced lower excretion rates. This strongly suggests that, in Cory's shearwater, mercury excreted into the plumage reflects more the current ingestion of mercury at the time of feather formation than mobilisation of mercury accumulated in internal stores during the inter-moult period. Moreover, low rates of excretion to plumage in birds dosed before the start of moult suggest little accumulation of the ingested mercury into proteins with fast turnover (March *et al.* 1983) to be released afterwards during moult. Such low rates presumably result from an important excretion into faeces, as observed in growing gull chicks (25% of the intake excreted in 30 days; Lewis & Furness 1991) and adult non-moulting quails (up to 85% over twelve weeks; Lewis & Furness 1993). Indeed, methylmercury conversion to inorganic mercury in the gut flora, with subsequent excretion in the faeces, is also the most important mechanism in humans and other mammals (Farris *et al.* 1993, Gearhart *et al.* 1995).

The skin has been almost ignored as an avian excretory pathway for mercury. The existence of this pathway is indicated by increases of mercury concentrations in fully grown feathers of experimental birds thought to result from exogenous contamination with mercury-containing exfoliated epidermal cells (March *et al.* 1983, this study). In this study, excretion rates of dietary mercury via the skin adsorbed to the contour plumage were estimated to represent 2 to 6% of the intake. Hence, whereas mercury burdens in feathers reflect chiefly uptake of methylmercury from the blood circulating to the follicle during feather formation (Chapter 7, this study), there is a potential contribution from adsorbed mercury resulting from excretion through the skin. The relative importance of the skin excretory pathway to the feather mercury burden increases with the level of exposure to mercury (March *et al.* 1983, this study), decreases with time after exposure (March *et al.* 1983) and presumably it is higher in contour feathers than feathers away from the body. Thereafter, rates of mercury excretion by endogenous incorporation into the plumage, feather's dose-response relationships and blood-feather partition coefficients should always be viewed in the light of a potential confounding effect resulting from the mercury adsorption pathway, which may inflate estimates, especially in short-term experimental studies using high doses.

9.4.5. Toxicity

Although assessment of sub-lethal methylmercury toxicity in Cory's shearwater was not an objective in the experimental design, this study provides one of the few approaches of the kind in wild birds. The working hypothesis, that the doses employed were below the methylmercury toxicity threshold for the species, is supported by all indicators of avian methylmercury poisoning considered (see Methods). Indeed, egg production and hatchability do not indicate acute toxicity over the dose-range, but a complete assessment would require an elaborate experimental design to control for the effect of breeding experience of experimental pairs on such breeding parameters. Moreover, despite experimental pairs being handled more frequently than controls they showed an identical overall hatching success. The lack of significant dose effects on body condition of adults and growth of chicks suggests a normal behavioural performance in both age classes and, again, absence of toxicity or disturbance effects.

Exposure levels used in this study ranged from 0.3 to 2.5 μg methylmercury/g of body mass and were well below the maximum reported avian non-observed-adverse-effect-level (NOAEL, 67 μg/g) for external symptoms, observed in adult Zebra finches subjected to a dietary level of 2.5 ppm dw over a 77 day period (Scheuhammer 1988). However, normal behavioural patterns of adult birds have been reported to be altered by dosages below those at which external symptoms of mercury poisoning are apparent, especially in young (reviews in Fimreite 1979, Scheuhammer 1987). Severe neurological signs of methylmercury poisoning develop in adult Zebra finches at brain concentrations of 15 ppm fw and over 30-40 ppm fw in liver and kidney, but young may develop such symptoms at brain levels four times lower (Scheuhammer 1988). The avian reproductive system seems to be the most prominent target of mercury poisoning. Impairments have been observed at egg concentrations in the range of 1-3 ppm (fw or dw basis is not specified) but some species are not affected even at higher levels (Fimreite 1979, Scheuhammer 1987). A field study by Barr (1986) indicated that egg laying and territorial fidelity were impaired in breeding loons *Gavia immer* at mercury concentrations of 0.3-0.4 ppm fw in prey, which constitutes the first lowest-observed-adverse-effect-level (LOAEL) for wild bird populations. Nevertheless, mercury toxicity in birds seems to be species-specific (Gardiner 1972) and the high concentrations found in fish-eating birds with apparently no ill or reproductive effects (Thompson & Furness 1989a, Becker *et al.* 1993) may be

related to co-accumulation of selenium which is known to antagonise the metabolic effects of mercury (Fimreite 1979, Scheuhammer 1987, 1988).

Avian and human methylmercury toxicity seems to differ markedly. Natural blood levels in Cory's shearwater (600 ppb fw or 2600 ppb dw) or Bulwer's petrel *Bulweria bulwerii* (2200 ppb fw or 11000 ppb dw; Chapter 7) are, respectively, 60 and 220 times higher than those in humans (10 ppb fw, WHO 1990). This cannot be accounted for solely from differences in the values of the principal kinetic parameters (half-time of the slow terminal phase and fractional deposition in blood) and presumably results from a much higher ponderal intake of mercury. It is noteworthy, that average blood levels of Cory's shearwater are 3 times above the blood concentration associated with a 5% risk of neurological damage to human adults (200 ppb fw, WHO 1990) and 10 times higher than blood concentration in pregnant women associated with a 5% risk of neurological damage to infants (60 ppb fw, WHO 1990). Cory's shearwater and birds in general exhibit a lower susceptibility to methylmercury toxicity than humans. This may result from the remarkable selectivity of methylmercury to the higher and evolutionary more recent structures of the brain such as the cortical areas. Indeed, vulnerability to methylmercury may not have been a problem throughout most of evolution, until recent times when the brain developed in mammals and especially primates (Clarkson 1994). Alternatively, the naturally high exposure of seabirds to mercury in the food chain may have led to the evolution of more powerful detoxification mechanisms in these birds (e.g. Thompson & Furness 1989a).

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CHAPTER 10

COMPARATIVE KINETICS, DOSE-RESPONSES, EXCRETION AND TOXICITY OF METHYLMERCURY IN FREE-LIVING SMALL AND LARGE CORY'S SHEARWATER CHICKS

toxicity in free-living young birds is still needed. In this respect, it is particularly relevant to gain some insight into the kinetics of methylmercury in blood, due to its role as internal carrier and ubiquous contact with other tissues.

This study constitutes the first comprehensive evaluation of kinetics, dose-response relationships and excretion of methylmercury in free-living growing birds. Small and large chicks were exposed to a single oral dose of methylmercury in two independent experiments and a non-destructive multi-tissue approach (using blood and plumage) was employed with the following objectives: (1) To determine the half-time of methylmercury in blood; (2) To derive relationships between steady-state dietary intake of methylmercury and blood concentrations; (3) To obtain partition coefficients for blood:plumage mercury concentrations; (4) To obtain dose-response relationships between acute methylmercury dietary intake and levels in blood and plumage; (5) To quantify the deposition of mercury into plumage; (6) To assess the potential toxicity of the doses administered; (7) To assess the effect of age at exposure on the results under all the former objectives.

10.2. METHODS

10.2.1. EXPERIMENTAL PROCEDURE

Cory's shearwater *Calonectris diomedea* was chosen as study-species for a variety of reasons: it is abundant; chicks are nidicolous and have a long development period; diurnal handling does not interfere with parental attendance, which is nocturnal. A calendar for fieldwork was set based on the patterns of growth and plumage development of the chicks. Growth takes three month between late July and late October, with three main phases: exponential growth (early August to early September), asymptotic growth (mid-September to early October), prefledging fasting (Granadeiro 1991, Sub-chapter 2.2). The development of plumage was as follows: chicks are covered by primary down at hatching; secondary down grows from the second week; the primary down falls off in the fifth week and in the eighth week the growing feathers were loosening the secondary down; primary wing coverts, ventral and dorsal feathers protrude in about the fourth week; tails protrude in the sixth to seventh week and primaries do so about the eighth; the plumage is fully grown at fledging, except for the last primaries in some individuals.

Two experiments -1 and 2- were undertaken at the colony on Vila Islet (Azores), in 1994, during five visits: 10-12 and 27-29 August, 10-12 and 24-25 September and 16-19 October. In each experiment, seven chicks were randomly assigned to each of three dose groups of 100 µg, 500 µg or 1000 µg. In addition, eight chicks randomly selected were used as controls for both experiments. The rationale for experiments 1 and 2 was to investigate age effects in relation to objectives 1 to 6 in the introduction. Therefore, chicks from experiment 1 were dosed in early August (1 to 2 weeks after hatching, i.e. during exponential growth) and chicks from experiment 2 were dosed in mid-September (6 to 7 weeks after hatching, i.e. during asymptotic growth). Chicks were ringed, weighed and measured (Sub-chapter 2.2). Single-doses of methylmercury were administered orally in the form of analytical grade methyl mercuric chloride solution placed in gelatine capsules. After dosing, the birds were released into their burrow. In experiment 1, the chicks were retrieved to collect blood samples of 0.5 to 2 ml (representing always less than 10% of the whole blood volume of the chick; Sturkie 1986) on days 2, 18, 32, 45 and 67 after dosing. The plumage was sampled on two occasions. On day 32, samples of secondary down (hereafter down 2) and growing ventral contour feathers were collected. On day 67, samples of naturally growing ventral contour feathers were collected along with ventral feathers induced on day 32. In experiment 2, the chicks were retrieved to collect blood samples of 1.5 to 2 ml on days 2, 14 and 36 after dosing. The plumage was sampled in two occasions. On day 0 a sample of growing ventral contour feathers was collected. On day 36, samples of naturally growing ventral contour feathers were collected along with the ventral feathers induced on day 0. Blood sampling was initiated forty-eight hours after dosing to allow complete absorption of the ingested dose and to avoid collection of samples with high transient concentrations (Sherlock *et al.* 1984). To assess the validity of this assumption, 3 chicks were dosed with 1000 µg along with those in experiment 2, and blood samples were collected at 12h, 24 h and 48 h post-dosing. Data from a feasibility pilot-study undertaken with large chicks, dosed with 1000 µg in 20-21 September 1993, were also employed to compare deposition of the dose in sections of feather's vane existing prior to dosing and formed after dosing.

The doses were chosen to double the range of exposures naturally experienced by the species, as estimated from levels in Atlantic and Mediterranean colonies (Renzoni *et al.* 1986). It was estimated that a dose of ~1000 µg was necessary to increase by nine the plumage burden of mercury (0.8 µg/g x 65 g;

based on a elimination rate of 49% to the plumage, Lewis & Furness 1991). The dose range employed was assumed to be sub-toxic, and data were collected to assess the potential earliest avian symptom of mercury poisoning in chicks, which is weight loss (Scheuhammer 1987).

10.2.2. Sample collection, preparation and mercury analysis

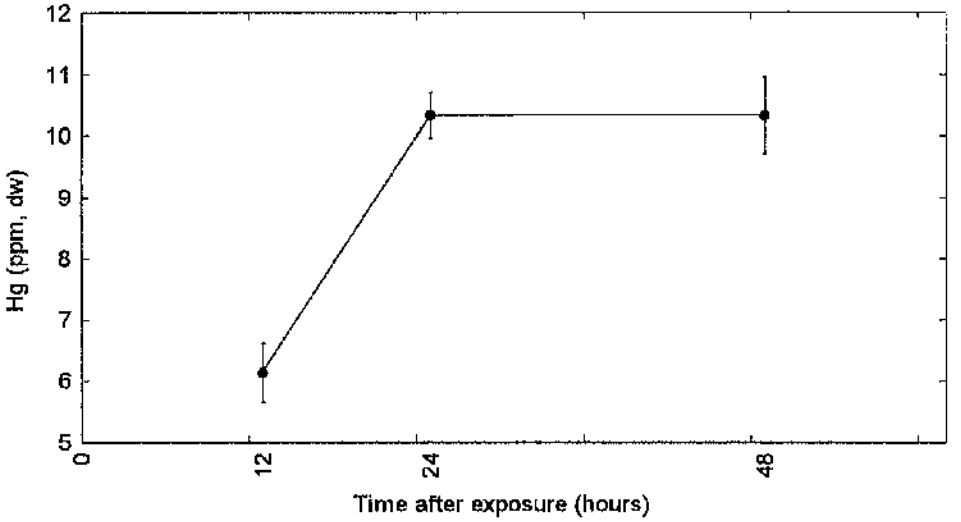
Sampling of plumage and blood, tissue preparation and storage, total mercury analysis of tissues and analytical quality control were carried out as described in Chapter 4. All concentrations are given in microgram per gram on a fresh weight (ppm, fw) or dry weight (ppm, dw) basis.

10.2.3. Assumptions and data analysis

In this study, the analysis of mercury kinetics relies on two main assumptions. The first is that the single dose of methylmercury solution will be handled in the same way as doses absorbed repeatedly from the ingestion of food. This may be the case, as several tracer studies with adult humans have shown an overall similarity in the kinetic parameters of methylmercury estimated from acute or chronic exposure via contaminated food (Kershaw *et al.* 1980, Sherlock *et al.* 1984). The second assumption is that concentration of total mercury in blood is a reliable indicator of changes due to the ingestion of methylmercury. Indeed, it was shown that concentrations of inorganic mercury did not change significantly in whole blood following single doses of methylmercury in humans (Kershaw *et al.* 1980) and in growing rats (Thomas *et al.* 1988).

Total mercury concentrations determined in individual samples of tissues from experimental groups were corrected by subtracting the mean concentration found in the corresponding samples from controls. All subsequent analysis and calculations use these 'net' mercury concentrations, except where otherwise stated, and follow procedures outlined in Chapter 4. Values of the variable time refer to days after complete absorption of the dose, therefore time 0 = day 2 after ingestion. One chick from the dose group 500 µg in experiment 1 did not increase in body mass between dosing and day 18 and was eliminated from the experiment on the assumption it had been deserted by its parents. The kinetics of ingested methylmercury in whole blood of the experimental chicks was analysed with pharmacokinetic models described in the appendix.

Fig. 10.1
Mercury concentrations (mean ±S.E.) in blood of chicks dosed with 1000 ug in September (n=3), sampled at three times within 48 h after exposure.



10.3. RESULTS

10.3.1. General

The body mass of Cory's shearwater chicks increases from less than 100g at hatching to a peak of 1200g at 9 weeks of age (Granadeiro 1991) and, hence, the doses administered resulted in variable loads of methylmercury in relation to chick age at exposure. The doses normalised to body mass (D_n, µg/g) averaged in experiments 1 and 2, respectively: 0.28 and 0.094 for the 100 µg dose group; 1.65 and 0.46 for the 500 µg dose group; 2.52 and 1.06 for the 1000 µg dose group.

Mean blood mercury concentrations at 12h, 24 h and 48 h post-dosing (Fig. 10.1) differ significantly between 12 h and 24 h but not between 24 h and 48 h (1-way ANOVA, $F_{2,6}=22.94$, $P<0.002$ plus Tukey tests). This supports the assumption that absorption was completed at time zero, i.e. 48 h after exposure.

TABLE 10.1. - Body mass (n = 20-21) and mercury concentrations in blood (µg/g dw; mean ±S.E.) in chicks from experiments 1 and 2, at various times after the absorption of the single oral dose. Mean values in bold and italic do not differ significantly from a mean of zero (t-tests, $P<0.05$).

Experiment	Time (days)	Body mass (g)	Hg in blood, Dose (µg)		
			100	500	1000
1	0	431 ±59	2.0 ±0.3	10.6 ±3.4	21.5 ±3.9
	16	857 ±133	0.3 ±0.05	1.4 ±0.2	3.4 ±0.3
	30	982 ±99	0.1 ±0.02	0.4 ±0.04	0.8 ±0.2
	43	1090 ±93	***-0.01 ±0.007***	0.1 ±0.02	0.2 ±0.02
	65	1057 ±91	-0.04 ±0.005	***0.1 ±0.09***	0.08 ±0.03
2	0	1044 ±127	1.0 ±0.06	4.9 ±0.3	10.6 ±0.4
	12	1130 ±108	0.2 ±0.02	1.2 ±0.09	2.2 ±0.2
	34	1086 ±113	***0.08 ±0.03***	0.6 ±0.08	0.8 ±0.1

10.3.2. KINETICS OF METHYLMERCURY IN BLOOD

10.3.2.1. Elimination

Table 10.1 shows the mean body mass and mercury concentrations in blood of chicks from experiments 1 and 2, at various time periods after dosing. In experiment 1, mercury was not detectable in the blood of the 100 µg and 500 µg dose groups after 43 and 65 days, respectively, but was still detectable at 65 days in the 1000 µg dose group. In experiment 2, mercury was still detectable at 34 days after dosing, except for the group dosed with 100 µg.

The blood methylmercury concentration profiles with respect to time were fitted by one compartment models in bivariate (10.1) and multi-dose (10.2) forms (see Appendix). Individual and common half-times of methylmercury in blood are given in Table 10.2, for each dose group in experiments 1 and 2. Individual estimates ranged from 3.6 to 7.7 days, without any significant effect of dose, either in experiment 1 (1-way ANOVA, $F_{2,17}=0.73$, $P=0.50$) or in experiment 2 (1-way ANOVA, $F_{2,18}=1.65$, $P=0.22$). Individual half-times did not differ significantly between the two experiments (pooled dose groups; $t_{40}=0.20$,

Fig. 10.2
Multi-dose model for decline of mercury in blood of Cory's shearwater chicks from experiment 1.

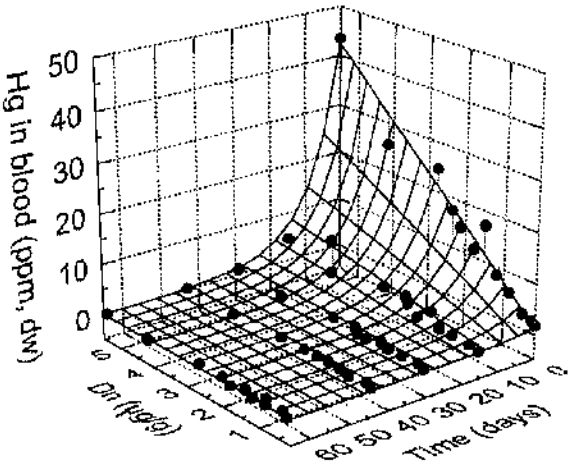
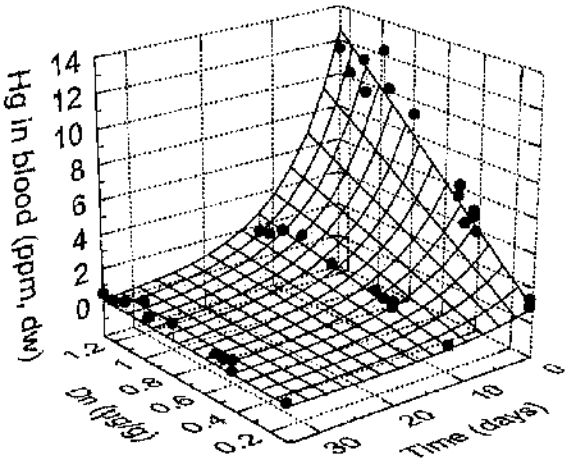


Fig. 10.3
Multi-dose model for decline of mercury in blood of Cory's shearwater chicks from experiment 2.



P=0.84). There is an overall close agreement between individual and common half-time estimates. The multi-dose model fits well the pooled-dose elimination profiles of methylmercury in blood of chicks from experiment 1 (r=0.98, n=101; Fig. 10.2) and experiment 2 (r=0.98, n=63; Fig. 10.3)

$$\text{Exp. 1:} \qquad C = Dn * 7.67 * e^{-0.117*t} \qquad (10.8)$$

$$\text{Exp. 2:} \qquad C = Dn * 10.01 * e^{-0.126*t} \qquad (10.9)$$

and produces overall estimates of the initial concentrations and half-times identical to those of the bivariate model (Table 10.2).

TABLE 10.2. - Kinetic parameters (*A*-initial concentrations, $T_{½}$-half-times, *f*-fraction of dose deposited in blood) for blood of Cory's shearwater chicks exposed to single oral doses of methylmercury in experiments 1 and 2. Values for individual estimates are mean ±S.E. Common estimates of *f* are based on the intercepts *A* obtained from bivariate and multi-dose models.

Experiment	Type of estimate	Model (equation)	Dose (μg)	*A* (μg/g)	$T_{½}$ (days)	*f*
1	individual		100	2.0*	5.9 ±0.4	0.104 ±0.007
			500	10.6*	5.9 ±0.5	0.105 ±0.013
			1000	21.5*	5.8 ±0.2	0.130 ±0.010
	common	bivariate	100	2.01	5.8	0.112
		(10.1)	500	10.56	5.8	0.120
			1000	22.14	5.7	0.146
		multi-dose	pooled	–	5.9	–
		(10.2)	100	2.12		0.121
			500	12.65		0.144
			1000	21.29		0.140
2	individual		100	1.0*	5.6 ±0.4	0.124 ±0.007
			500	4.9*	6.3 ±0.3	0.117 ±0.007
			1000	10.6*	5.5 ±0.3	0.127 ±0.006
	common	bivariate	100	1.03	5.5	0.124
		(10.1)	500	4.90	6.2	0.118
			1000	10.60	5.4	0.127
		multi-dose	pooled	–	5.5	–
		(10.2)	100	0.94		0.113
			500	4.60		0.110
			1000	10.58		0.127

* Values taken from Table 10.1, for time 0.

10.3.2.2. Intake and equilibrium concentrations

A dose-response relationship for equilibrium methylmercury concentrations in blood of Cory's shearwater chicks was derived using equations (10.4) through (10.7) in the Appendix. Blood volume was assumed to represent 7% of body mass (Sturkie 1986) for chicks of experiment 1. For chicks of experiment 2 (all with body mass >900 g at dosing) the average adult blood volume of 60 ml (i.e. 12 g on a dry weight basis) was used, since the excess of mass comparatively to adults is a result of temporary accumulation of fat.

Individual and common estimates of the fraction of dose deposited in the blood volume (*f*) are given in Table 10.2. Individual estimates ranged from 0.077 to 0.162, without any significant effect of dose, either in experiment 1 (1-way ANOVA, $F_{2,17}=2.03$, $P=0.16$) or in experiment 2 (1-way ANOVA, $F_{2,18}=0.63$, $P=0.54$). Individual estimates did not differ significantly between the two experiments (pooled dose groups; $t_{40}=1.37$, $P=0.18$). There is an overall close agreement between individual and common half-time estimates, which differ by less than 15% (except for the 500 µg dose group in exp. 1). An average *f* of 0.12 and half-time of 5.7 days were employed in equation (10.7) to derive a relationship between steady state methylmercury concentration in blood (*C*, µg/g fw) and daily intake (*I*, µg/day) for a given body mass (*W*, g)

$$C = I \,.\, (14.1 \,/\, W) \qquad (10.10)$$

10.3.3. Blood:plumage relationships

Mercury concentrations in blood samples collected simultaneously with plumage underestimate the actual average blood concentration during the period of plumage formation due to the pattern of rapidly declining blood levels after exposure (cf. Table 10.1). Thus, mercury concentrations in the plumage of Cory's shearwater chicks were regressed against individual average blood concentrations during the respective period of plumage formation. The details of calculation of such average concentrations are given in Table 10.3.

Blood:plumage relationships conform to a linear model for all plumage types in both experiments and are summarised in Table 10.3. The blood-naturally growing ventral feather relationships did not differ significantly between the two sampling dates in experiment 1 (ANCOVA, $F_{1,37}=0.091$, $P=0.76$) but differences between experiment 1 (pooled data from the two periods) and experiment 2 were highly significant (ANCOVA, $F_{1,58}=29.57$, $P<0.0001$). The blood:induced ventral

TABLE 10.3. - Summary of the linear regressions between mercury concentrations in plumage (PL, µg/g fw) and blood (BL, µg/g dw) of Cory's shearwater chicks from experiments 1 and 2. The general equation of lines constrained to pass through the origin was PL = b * BL. Codes for plumage types are: D2, down 2; NGVF, naturally growing ventral feathers; IVF, induced ventral feathers).

Plumage		Experiment	b ±S.E.	Statistics
type	sampling (days post-dosing)			r, F*
D2	32 [a]	1	2.6 ±0.3	$r=0.91$, $F_{1,19}=87.10$
NGVF	32 [b]	1	5.6 ±0.4	$r=0.95$, $F_{1,19}=195.8$
	67 [c]		5.6 ±0.8	$r=0.85$, $F_{1,19}=50.35$
	36 [d]	2	2.2 ±0.1	$r=0.97$, $F_{1,20}=350.3$
IVF	67 [e]	1	2.0 ±0.3	$r=0.87$, $F_{1,19}=59.38$
	36 [d]	2	1.1 ±0.05	$r=0.98$, $F_{1,20}=456.7$

* $P<0.0001$ for all regressions.

Lower case superscripts refer to sampling periods (in days post-dosing) used to estimate the average blood concentration during plumage formation: [a] 0,17,32; [b] 17,32; [c] 17,32,45,67; [d] 0,14,36; [e] 32,45,67.

feather relationships did not differ significantly between experiments 1 and 2 (ANCOVA, $F_{1,38}=0.75$, $P=0.39$). Scatterplots of mercury concentrations in blood and naturally growing or induced ventral feathers are shown, respectively in Figures 10.4 and 10.5.

10.3.4. Dose-response relationships

Blood mercury concentrations after complete absorption of the single oral dose (i.e. 48 h after exposure) increased linearly with dose normalised for body mass (D_n) (Exp. 1: $r=0.98$, $F_{1,19}=397.8$, $P<0.0001$; Exp. 2: $r=0.99$, $F_{1,20}=1111.2$, $P<0.0001$; Fig. 10.6), without significant differences between experiments (ANCOVA, $F_{1,39}=1.64$, $P=0.21$):

$$\text{Exp. 1:} \quad Hg = 8.87 \pm 0.44 * Dn \qquad (10.11)$$

$$\text{Exp. 2:} \quad Hg = 10.03 \pm 0.30 * Dn \qquad (10.12)$$

Fig. 10.4
Scatterplot of mercury concentrations in naturally growing ventral feathers (ppm, fw) and blood (ppm, dw) for chicks in experiments 1 and 2.

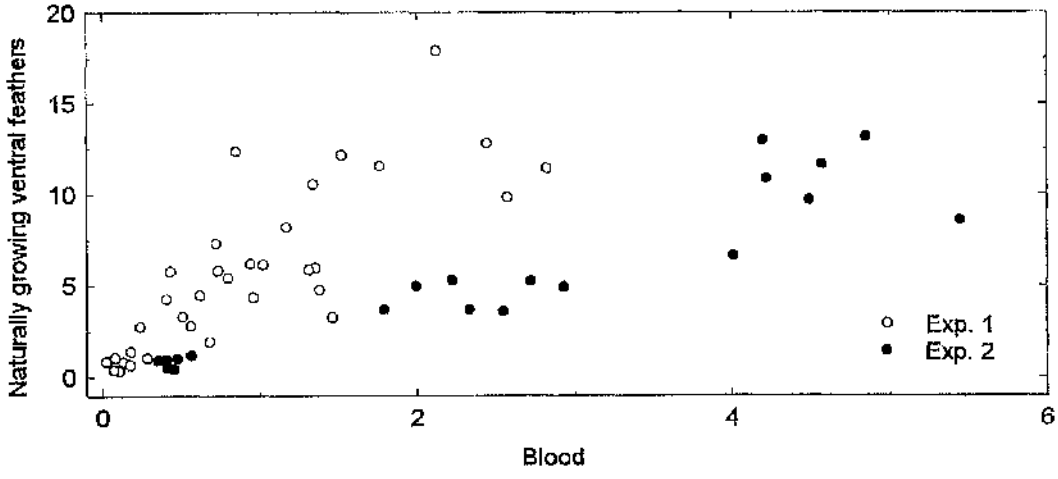


Fig. 10.5
Scatterplot of mercury concentrations in induced ventral feathers (ppm, fw) and blood (ppm, dw) for chicks in experiments 1 and 2.

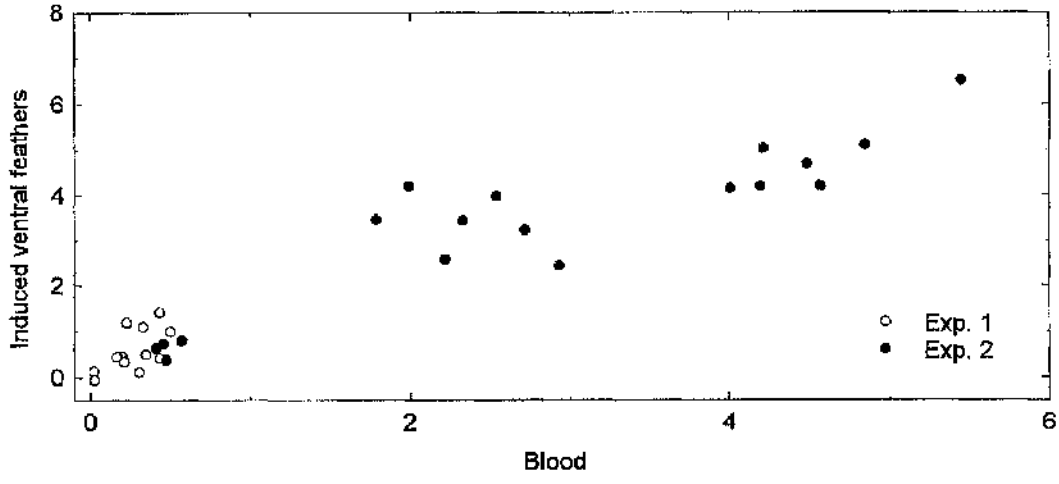
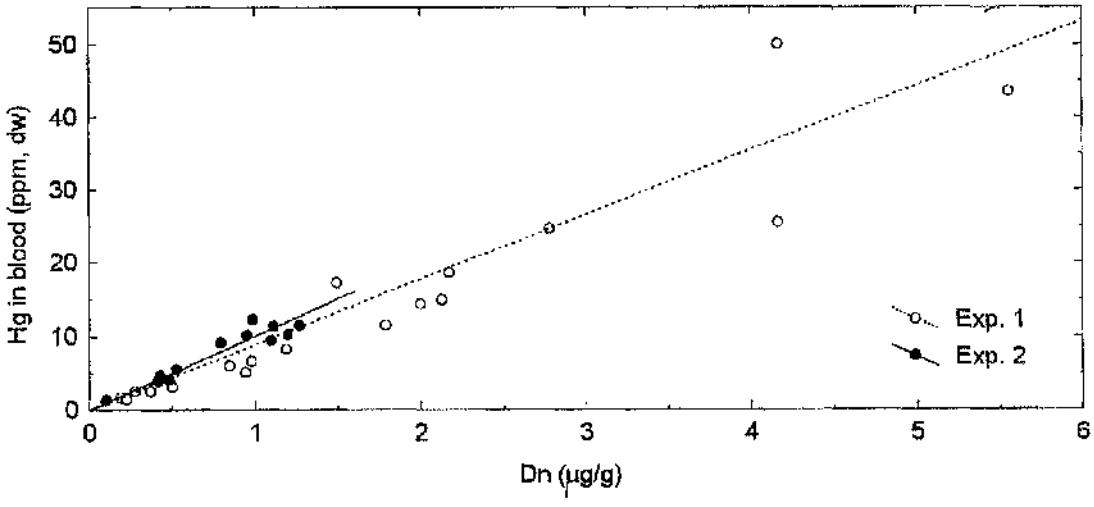


Fig. 10.6
Dose-response relationship for blood mercury concentration after exposure, for chicks in exps. 1 and 2. Fitted lines constrained to pass through the origin.



Mercury concentrations in different plumage types collected at various periods after exposure from chicks of experiments 1 and 2 and the linear dose-response relationships observed between such concentrations and dose normalised to the initial body mass (D_n) are given in Table 10.4.

10.3.5. Excretion

Mercury intake was excreted into the secondary down of chicks in experiment 1, as indicated by dose-response relationships in Table 10.4. However, an estimate of the amount excreted in this way is precluded by a lack of information on the mass of secondary down formed. Hereafter, excretion rates refer to the feathers which form the final plumage and were common estimates derived by multiplying a mean plumage mercury concentration by a mean plumage mass of 65 g (Monteiro unpubl. data). Mercury concentrations in pre-fledglings did not vary significantly among feather types (Table 10.5) and concentrations in ventral feathers were used as an indication of the mean plumage concentration in experimental birds. Estimates of individual rates were precluded due to difficulty in obtaining accurate estimates of individual plumage mass in chicks with large fat deposits. Excretion rates estimated in this way, account for incorporation due to mercury uptake from blood during feather formation (Chapter 9, this study) and for external adsorption of mercury contaminated exfoliated epidermal cells due to excretion through skin (March *et al.* 1983, Chapter 9). In addition, mercury concentrations in primary 10 of chicks a month after administration of a single oral dose of 1000 µg of methylmercury in late September 1993, indicate that excretion is homogeneous between a distal section with 3.5 to 6.5 cm at dosing and a proximal section formed after exposure (µg/g fw, mean $\pm$S.E.; 10.4 $\pm$1.9 vs. 10.9 $\pm$3.4; t-test for dependent samples, $t_4 = 0.11$, $P = 0.91$).

Estimates of excretion into plumage ranged two-fold, from 35.1% to 68.1% (Table 10.6), with differences between experiments 1 and 2 approaching significance (Mann-Whitney test, $Z = 1.96$, $P = 0.05$).

10.3.6. Toxicity

Potential dose-induced toxicity was assessed by comparison of pre-fledgling body condition (as indicated by body mass) and chick growth among dose groups and controls. There was no significant effect of dose (controls included) on chick body

TABLE 10.4. - Mercury concentrations in different plumage types (codes are: D2-down 2, NGVF-naturally growing ventral feathers, IVF-induced ventral feathers; values are mean ±S.E., µg/g fw) sampled at various periods after exposure, for Cory's shearwater chicks of experiments 1 and 2 by dose group, and parameters for the linear regression between mercury concentrations in plumage (PL, µg/g fw) and dose normalised to body mass at exposure (Dn, µg/g) , for the same chicks. The general equation of fitted lines constrained to pass through the origin was PL = b * Dn.

Plumage		Expe-	Hg in plumage by dose group			Dn	
type	sampling (days post-dosing)	riment	100 µg	500 µg	1000 µg	b ±S.E.	Statistics: r, F
D2	32	1	3.1 ±0.6	14.9 ±2.5	27.0 ±4.8	9.1 ±0.8	$r=0.94$, $F_{1,19}=145.9$***
NGVF	32	1	1.2 ±0.3	5.4 ±0.3	12.3 ±1.0	3.3 ±0.5	$r=0.83$, $F_{1,19}=41.97$***
	67		0.5 ±0.08	3.6 ±0.5	6.8 ±1.1	1.6 ±0.4	$r=0.68$, $F_{1,19}=16.75$***
	36	2	0.8 ±0.1	4.5 ±0.3	10.5 ±0.9	9.9 ±0.3	$r=0.99$, $F_{1,20}=1281.9$***
IVF	67	1	0.04 ±0.03	0.7 ±0.1	0.6 ±0.2	0.2 ±0.05	$r=0.95$, $F_{1,19}=13.56$**
	36	2	0.6 ±0.06	3.3 ±0.2	4.8 ±0.3	4.9 ±0.3	$r=0.96$, $F_{1,20}=227.4$***

** $P<0.005$, *** $P<0.0001$.

TABLE 10.5. - Comparison of absolute mercury concentrations (not corrected for controls) among feather types of pre-fledgling controls and chicks dosed with 1000 µg in experiment 2. Values are mean ±S.E.

Feather type	Hg (µg/g, fw)	
	controls	Dose 1000 µg
ventral	0.62 ±0.02	7.52 ±1.84
primary 1	0.76 ±0.08	-
primary 10	0.69 ±0.10	7.99 ±2.49
outer rectrix	0.75 ±0.07	-
	$F_{3,16}$=0.67*, P=0.58	t_4=0.12**, P=0.91

*1-way ANOVA, **t-test for dependent samples.

TABLE 10.6. - Estimates of mean plumage mercury concentration (mean ±S.E.) and excretion into plumage in pre-fledgling chicks from experiments 1 and 2, by dose group.

Experiment	Dose (µg)	mean Hg in plumage* (µg/g, fw)	Total Hg excretion (µg)	Total Hg excretion as % of intake
1	100	0.54 ±0.08	35	35.1
	500	3.57 ±0.54	232	46.4
	1000	6.84 ±1.11	445	44.4
2	100	0.84 ±0.10	55	54.6
	500	4.47 ±0.30	291	58.1
	1000	10.48 ±0.90	681	68.1

*As inferred from mean concentration in normally growing ventral feathers.

TABLE 10.7. - Parameters (mean ±S.E.) for the quadratic function of body mass (BM) on wing length (WL) fitted to data of individual chicks from experiment 1.

Dose (μg)	n	Parameters for growth curve $BM = A + B*WL + C*WL^2$		
		A	B	C x100
0	8	214 ±43	6.6 ±0.7	-1.2 ±0.2
100	7	46 ±37	8.8 ±0.4	-1.8 ±0.1
500	6	49 ±43	8.8 ±0.7	-1.8 ±0.2
1000	7	98 ±71	9.0 ±1.6	-2.0 ±0.6
1-way ANOVA		$F_{3,24}=2.57$, $P=0.078$	$F_{3,24}=1.39$, $P=0.27$	$F_{3,24}=1.06$, $P=0.38$

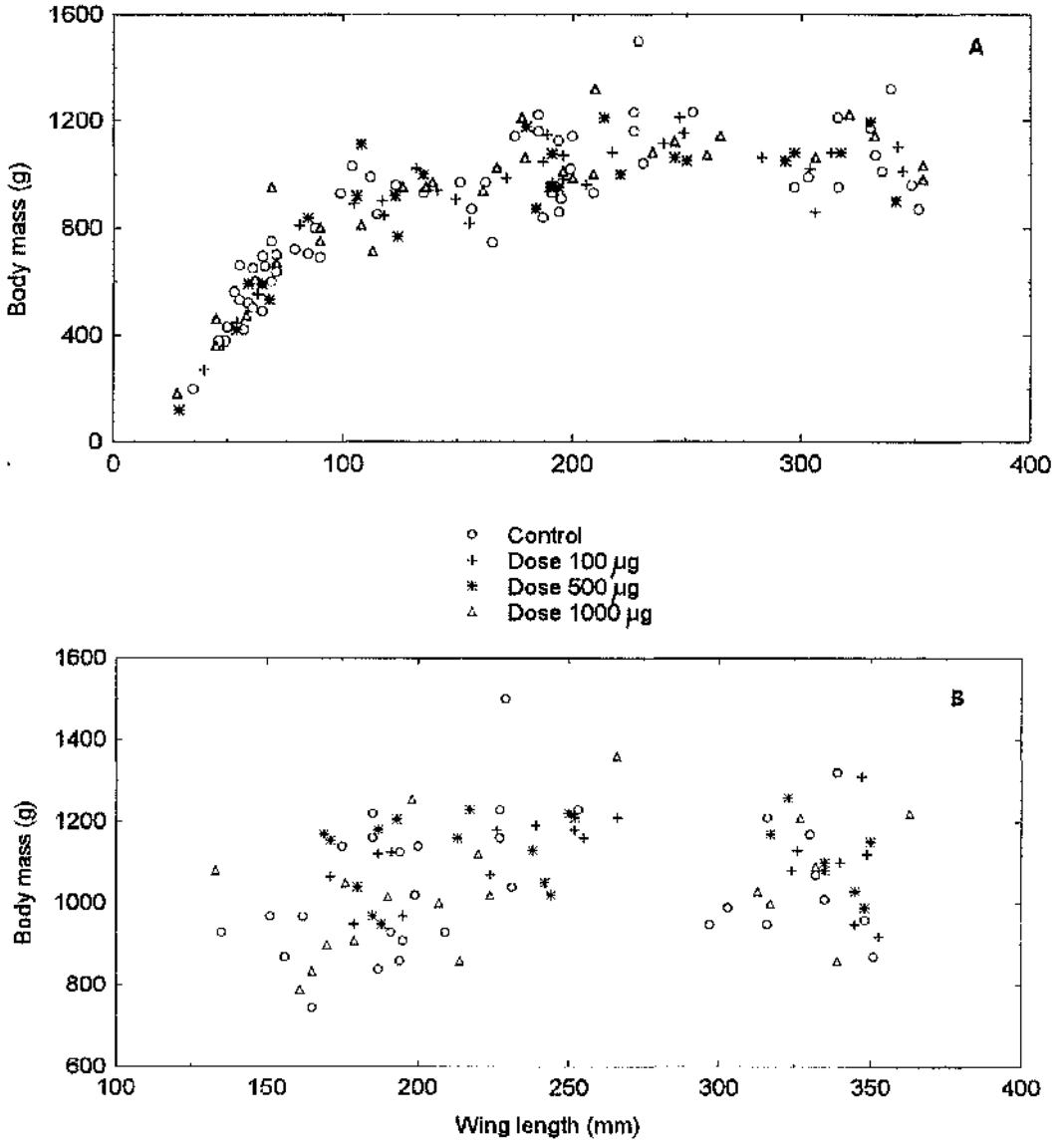
mass measured within two weeks before fledging, both in experiment 1 (ANCOVA, $F_{2,16}=0.81$, $P=0.46$; wing length as covariate) and in experiment 2 (ANCOVA, $F_{2,17}=0.35$, $P=0.71$; wing length as covariate). In addition, parameters of growth curves in the form of a quadratic function of body mass on wing length, fitted to data from individual chicks from experiment 1, did not differ significantly among dose groups and controls (Table 10.7). This analysis was not undertaken with chicks from experiment 2 due to the small number of data points ($n=3$) to fit a reliable growth curve over a period of peak body mass and pre-fledgling weight loss. The scatter of pairs body mass-wing for controls and experimental birds is shown in Fig. 10.7 for experiments 1 and 2.

10.4. DISCUSSION

10.4.1. KINETICS OF METHYLMERCURY IN BLOOD

The time profiles of methylmercury elimination in blood modelled in this study were assumed to represent only the slow terminal phase. The highly transient concentrations from the initial fast phase (e.g. Kershaw *et al.* 1980, Sherlock *et al.* 1984) were thought to be extinguished between absorption, which was completed 24 h after exposure, and blood sampling at 48 h post-exposure. Indeed,

Fig. 10.7
Scatterplot of body mass and wing of controls
and experimental chicks from experiments 1 (A) and 2 (B).



chicks exhibit an half-time for the terminal phase much shorter than adults (this study, Chapter 9) and the same is conceivable for the half-time in the initial phase, which is less than 1 day for adults (Chapter 9).

Average estimates of blood methylmercury half-times in the terminal elimination phase ranged from 5.5 to 6.3 days, indicating a remarkable overall resemblance in all dose groups from both experiments. To allow an higher degree of confidence on these estimates it would have been desirable to have had better coverage over the 10 day period following the start of blood sampling.

Comparative half-time estimates in adult Cory's shearwater ranged from 40 to 65 days (Chapter 9). Such a difference in half-times between chicks and adults indicates the importance of the rapid growth of body tissues and plumage in governing mercury dynamics in chicks. Chicks have an opportunity to excrete methylmercury much faster than adults. It is proposed that such short half-times are mainly accounted for by elimination into growing tissues, in experiment 1, and by elimination into growing plumage, in experiment 2. The role of plumage renewal on the elimination kinetics of mercury in blood is stressed by a 30% reduction in half-time observed between adult Cory's shearwater exposed a month and two months and an half before the start of moult (Chapter 9). Other comparative avian blood half-times are not available in the literature. Half-times of methylmercury in richly perfused tissues (as liver) of growing chickens ranged from 4.1 to 6.8 days, over a wide range of exposure levels, and were shorter than in adult chickens (March *et al.* 1983). Half-time in blood of growing rats, estimated by fitting a one compartment model to data from Thomas *et al.* (1988), was 3.1 days.

The estimate of the fraction of ingested methylmercury deposited in the blood volume of experimental Cory's shearwater chicks averaged 0.12, without significant variations among dose groups or between age at exposure. A comparative estimate in adult Cory's shearwater was slightly lower (0.104; Chapter 9) and that variation could be accounted for by a higher percentage of fat in chicks comparatively to adults, leading to overestimates of the blood volume in chicks and hence of *f*. The knowledge of methylmercury terminal half-time in blood and of the fractional deposition of the ingested dose in blood, allowed a first insight into the relationship between steady-state blood concentrations and dietary intake of methylmercury in avian chicks. Based on a crude estimate of daily food consumption of 100 g (Klomp & Furness 1992, Hamer & Hill 1993) with an average of 16 ppb fw of methylmercury (Sub-chapter 8.2), the equilibrium concentrations in blood estimated from equation (8) agree closely with those observed under natural conditions (Chapter 7), for the body mass range covered in this study (400 to 1200 g). This suggests that the estimates of kinetic parameters for methylmercury in blood of Cory's shearwater chicks obtained in this single intake study are representative of those for repeated ingestion under natural conditions. It is also noteworthy that kinetic parameters for methylmercury in blood were independent of the phase of growth (exponential or asymptotic) at which they were exposed.

10.4.2. Blood:plumage partition coefficients

The slopes of the linear blood:plumage relationships obtained here represent estimates of the levels of mercury which partitioned into plumage of avian chicks. Since plumage contains virtually no inorganic mercury (Thompson & Furness 1989b, Sub-chapter 11.2), such slopes may well represent methylmercury partition coefficients. The range of slopes observed in this study (1.1 to 5.6) is slightly wider comparatively to the ranges observed for the same species in experimental adults (2.9 to 4.3; Chapter 9) and in adults and chicks under natural conditions (2.3 to 3.4; Chapter 7). However, most of the variation might be accounted for by difficulties in estimating accurately the average blood concentration during plumage formation, due to highly transient declining concentrations (cf. Table 10.1). The linearity of blood:plumage relationships observed indicate a lack of dose-dependence for the partition coefficients.

10.4.3. Dose-responses

The dose-response relationships determined in this study were linear over the wide range of exposures employed. That contributes additional evidence to a lack of saturation in the metal-accumulatory ability of blood (also Chapter 9), plumage (also Lewis & Furness 1991, Chapter 9) and other avian tissues currently used as monitoring units, like eggs (Tejning 1967, Chapter 9) and internal tissues (Lewis & Furness 1991, Scheuhammer 1988). There is a remarkable resemblance of dose-responses for blood among small and large chicks (cf. Fig. 10.6, this study) and adults (cf. Fig. 9.6, Chapter 9) of Cory's shearwater. That indicates an apparent general dose-response kinetics for methylmercury in avian blood, which seems to be governed only by the volume of the body pool, given that the fraction deposited in blood after complete absorption would not differ among species.

Dose-response relationships varied widely among the various plumage types of experimental chicks. The observed variation seems to arise essentially from declining blood levels in the chicks, since the earlier the time of plumage formation, the higher the response. For example, down 2 (exp. 1) and naturally growing ventral feathers (exp. 2), which were starting formation at dosing, attained a similar response within a month after exposure (cf. Table 10.4). There are no comparable data in the literature, as other avian studies did not report concentrations normalised to body mass.

10.4.4. EXCRETION

A discussion of the results under this topic requires a brief overview about the mechanisms of mercury excretion in birds. Growing plumage and faeces are major eliminatory pathways (e.g. Braune & Gaskin 1987b, Lewis & Furness 1991, Chapter 9), although excretion through the skin (followed by adsorption on feather surface) and eggs may also be of importance (March *et al.* 1983, Lewis & Furness 1993, Chapter 9). Whereas mercury burdens in feathers reflect chiefly uptake of methylmercury from the blood circulating to the follicle during feather formation (Chapter 7, Chapter 9), the contribution from adsorbed mercury resulting from excretion through the skin should be considered, especially in short-term experimental studies (Chapter 9). Data reported here suggest that mercury bound over the whole extension of the vane while the feather is in formation.

Overall, excretion rates into the final plumage estimated here averaged 42% and 60% for experiments 1 and 2, respectively. The difference was not statistically significant and in reality it is even less pronounced, since mercury excretion into secondary down of chicks from experiment 1 was not considered. Such excretion rates are in agreement with those reported in the literature for chicks growing an entire plumage (49%, Lewis & Furness 1991) and for adults during a moult cycle (59-68%, Braune & Gaskin 1987). When interpreting excretion rates into plumage by experimental birds (Lewis & Furness 1991, Chapter 9, this study) it should be noted that they did not account for mercury elimination into feather sheaths (Burger *et al.* 1992), but reflect to a certain extent (possibly up to 5-10%; Chapter 9) external feather contamination with exfoliated cells (March *et al.* 1983, Chapter 9).

The resemblance of average excretion rates in experiments 1 and 2 contrasts sharply with the enormous differences of blood mercury concentrations in the respective experimental chicks, in mid-September, when the final plumage is developing. The rate of excretion attained by chicks from experiment 1 suggest the existence of important short-term internal stores of mercury from which mercury was mobilised and eliminated into the final plumage, which started to develop 2 to 3 weeks after dosing. Results from experiment 1 indicate a lack of dose-dependency of plumage excretion rates, as in feathers and tissues of gull chicks exposed to low doses of methylmercury (Lewis & Furness 1991). However, there was a tendency for increasing excretion with dose in experiment 2, which may be accounted for by enhanced external contamination of the growing plumage with exfoliated epidermal cells (Chapter 9) in the higher dose group.

Methylmercury conversion to inorganic mercury in the gut flora, with subsequent excretion in the faeces, yet unquantified, is likely to have been an important excretion route in the experimental chicks from this study. Mercury excretion in faeces accounted for the excretion of 25% of the intake in growing gull chicks, over a 30 days period (Lewis & Furness 1991) and was the major excretory pathway in growing rats (Thomas *et al.* 1998, Farris *et al.* 1993).

10.4.5. Toxicity

Although assessment of sub-lethal methylmercury toxicity in Cory's shearwater chicks was not a primary objective in the experimental design, this study provides one of the few approaches of the kind in wild birds. The hypothesis was that the dose range employed was below the methylmercury toxicity threshold for the species. This is supported by the lack of significant dose effects on pre-fledgling body condition and growth of chicks, which were indicative of a normal behavioural performance.

Average exposure levels used in this study ranged from 0.09 to 2.5 μg methylmercury/g of body mass and compare with a maximum avian non-observed-adverse-effect-level (NOAEL) for external symptoms of 67 μg/g in adult Zebra finches *Poephila guttata* subjected to a dietary level of 2.5 μg/g dw over 77 days (Scheuhammer 1988). However, normal behavioural patterns of birds have been reported to be altered by dosages below those at which external symptoms of mercury poisoning are apparent, especially in young (reviews in Fimreite 1979, Scheuhammer 1987). Severe neurological signs of methylmercury poisoning develop in adult Zebra finches at brain concentrations of 15 μg/g fw and over 30-40 μg/g fw in liver and kidney, but young birds may develop such symptoms at brain levels four times lower (Scheuhammer 1988). The highest estimated average brain concentrations in experimental chicks from this study was 3.4 μg/g fw, based on a brain-blood ratio of mercury concentrations equal to 0.78 derived from concentrations in adult Cory's shearwater from Atlantic colonies (brain: 1.95 μg/g dw, Renzoni *et al.* 1986; blood: 2.5 μg/g dw, Chapter 7). This approaches close to the threshold for toxicity in young birds suggested by Scheuhammer (1988) and may well represent a maximum NOAEL for external symptoms in avian chicks. Nevertheless, it should be considered that mercury toxicity in birds is species-specific (Scheuhammer 1987). Fish-eating birds seem to support high mercury burdens with apparently no ill or reproductive effects perhaps due to co-

accumulation of selenium which is known to antagonise the metabolic effects of mercury (Fimreite 1979, Scheuhammer 1987 and 1988).

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APPENDIX - PHARMACOKINETIC MODELS

ELIMINATION OF MERCURY IN BLOOD

The kinetics of ingested methylmercury in whole blood of the experimental chicks was modelled according to current models for endotherm vertebrates (review in WHO 1990). This study showed that absorption of the single-oral dose was completed within 24 h after dosing (cf. Fig. 10.1) and it was assumed that the initial phase of rapid decline was completed at 48 h after dosing. Thereafter, the concentration of mercury $C(t)$ (µg/g) at time t (days) during the terminal elimination phase, which is the relevant in the assessment of equilibrium concentrations (Kershaw *et al.* 1980, Sherlock *et al.* 1984), is given by the one compartment model

$$C(t) = A \cdot e^{-\alpha \cdot t} \qquad (10.1)$$

where A (µg/g) and α (1/day) are unknown parameters to be estimated on the basis of experimental data. Given that the dose-response of methylmercury in blood is linear (Chapter 9; this study, section 10.3.4), a multi-dose version of model (10.1) may be derived by incorporating the methylmercury dose normalised to body mass (D_n, µg/g),

$$C(t) = D_n \cdot A \cdot e^{-\alpha \cdot t} \qquad (10.2)$$

In both models the half-time ($T_{½}$, days) is given by

$$T_{½} = (\ln 2) / \alpha \qquad (10.3)$$

INTAKE AND EQUILIBRIUM BLOOD CONCENTRATIONS

There is evidence that the relationship between the steady state concentration of methylmercury in blood (C, µg/kg) and the daily intake (I, µg/day) is identical in single-dose and chronic dosing studies (Sherlock *et al.* 1984) and that is given by (Kershaw *et al.* 1980):

$$C = I \,.\, (f / V) / \alpha \qquad (10.4)$$

where f is the fraction of the daily intake deposited in blood, V is the volume of blood (ml, i.e. g) and $\alpha = (\ln 2) / T_{½}$ is estimated from models (10.1) or (10.2). The parameter f is given by

$$f = V \,.\, A / D \qquad (10.5)$$

where A, the intercept of the slow component estimated from models (10.1) or (10.2), represents the blood concentration that would have resulted if absorption and tissue distribution were complete at time zero (Kershaw *et al.* 1980), and D is the dose (µg).

Assuming the blood volume is 7% of the body mass (W, g; Sturkie 1986),

$$C = I \,.\, (f \,.\, T_{½}) / (0.07 \,.\, \ln 2 \,.\, W) = I \,.\, a \qquad (10.6)$$

where a (days/g) represents the number of days intake of methylmercury contained in 1 g of circulating blood and is given by

$$a = (f / 0.07) \,.\, (T_{½} / \ln 2 \,.\, W) \qquad (10.7)$$

CHAPTER 11

SEABIRDS AS MONITORS OF MERCURY CONTAMINATION IN THE PORTUGUESE ATLANTIC

CONTENTS

Sub-chapter 11.1

CONTEMPORARY SPATIAL PATTERNS AND A META-ANALYSIS FOR THE NORTH ATLANTIC

11.1.1. INTRODUCTION

Mercury is a highly toxic non-essential metal of major concern for its harmful environmental effects because elevated exposures in Humans and wildlife tend to be a widespread phenomenon (UNEP/FAO/WHO 1987, WHO 1990). This derives from particular biogeochemical properties (i.e. evasion to the atmosphere coupled with long-range transport and deposition; EPMAP 1994) and mercury behaviour in aquatic ecosystems (e.g. methylation, bioaccumulation and bioamplification; Lindqvist *et al.* 1991).

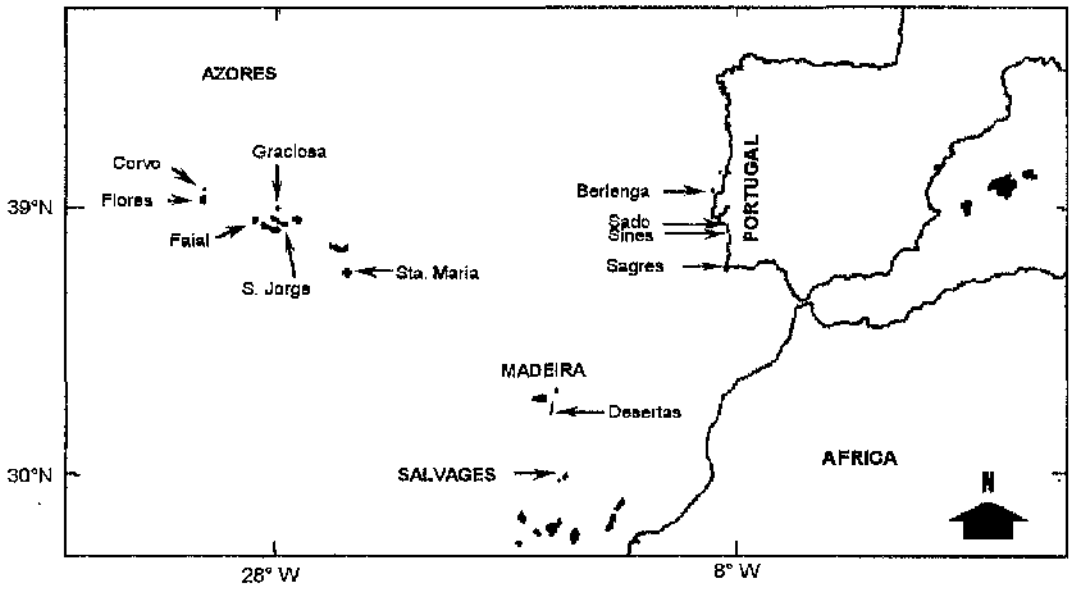
The current view of the overall global mercury cycle is well established, although a detailed understanding of many mechanisms is lacking (Mason *et al.* 1994a, EPMAP 1994). The cycle is dominated by emissions of mercury to the atmosphere either by natural processes or by human activities (reviews in Mason *et al.* 1994a, EPMAP 1994). Most atmospheric mercury (>95%) is in the elemental form, which has a long residence time (0.7-2 yr.; Lindqvist *et al.* 1991) and therefore is susceptible to long-range transport. Present direct anthropogenic emissions (chiefly from fossil fuel combustion and waste incineration) average about 50-75% of the total emissions to the atmosphere (EPMAP 1994). The oceanic biogeochemical cycle plays a predominant role in the global mercury cycle because of air-sea exchange processes (Mason *et al.* 1994b). Currently the oceans receive about 90% of mercury input through atmospheric deposition, while oceanic evasion (which is positively correlated with primary productivity; Mason *et al.* 1994b) contributes over two thirds of the total natural atmospheric emission (Mason *et al.* 1994a). Because the atmosphere and the oceans are in rapid equilibrium, a major proportion of contemporary oceanic effluxes are actually a re-emission of deposited anthropogenic mercury (EPMAP 1994). Such recycling of mercury between the surface oceans and the atmosphere, coupled with the small mercury sedimentation in the oceans, makes deposition on land the dominant sink for atmospheric mercury. Nevertheless, some processes rise above the global cycle. Ecological processes are responsible for enhanced methylmercury formation and bioaccumulation in sub-thermocline waters (Mason & Fitzgerald 1993, Sub-chapter 8.2). Local- and regional-scale processes also may account for a significant part of total atmospheric deposition of mercury inland (Nater & Grigal 1992, Sorensen *et al.* 1994) through dry deposition and precipitation scavenging of the small fraction (<5%) of atmospheric particulate mercury, which has a relatively short residence time in the atmosphere (Fitzgerald *et al.* 1991, Lindqvist

1994). Mass balances indicate that half of the antropogenically-emitted atmospheric mercury is removed in this way (EPMAP 1994).

This study covers a wide sector of the temperate and sub-tropical north Atlantic east of the Azores (30-40°N, 8-32°W). The region is characterised by highly dynamic oceanographic, atmospheric and geological processes, namely the north Atlantic gyre and the wind fields which overlie it, the Azores front, the upwelling off the Iberian Peninsula, the Canary Is. current, volcanism associated with the expansion of the mid-Atlantic ridge (Kennett 1982, Gould 1985, Harvey 1985, Pollard & Pu 1985). Most of the region represents a sub-tropical sector remote from continental anthropogenic emissions of mercury from which the information on the extent of mercury contamination is scanty. This study aims to meet some of the current research needs on mercury, namely to broaden the spatial and ecological representativeness of mercury records at pristine oceanic sites in order to shed light into the relative importance of local, regional and global mercury cycling processes (EPMAP 1994).

Because the behaviour of mercury in marine ecosystems is complex and includes many biologically-mediated processes, biomonitoring was preferred to the use of abiotic samples for direct determination of spatial patterns of mercury contamination in the study region (c.f. Phillips 1980). Species at the highest trophic levels integrate mercury contamination over food webs and will reflect better the environmental abundance and bioavailability of mercury and its hazards at the ecosystem level and to Humans. Seabirds offer varied levels of ecological, spatial and temporal integration and have been used as cost effective and successful monitors of mercury contamination in the marine environment (reviews in Furness 1993, Monteiro & Furness 1995). They excrete dietary mercury in a linear dose-dependent fashion into eggs and plumage (Chapters 9 and 10) and, therefore, feathers and eggs provide easily-obtainable and relatively non-invasive monitoring tools. However, the use of seabirds as monitors is not entirely straightforward and requires a good knowledge of their ecology and potential 'noise' from confounding factors. Such aspects were addressed in Sub-chapter 2.2, Chapters 5 , 6 and 7, and Sub-chapter 8.2. Here, mercury concentrations in tissues of breeding seabirds were used to infer contemporary regional patterns in mercury contamination within the Portuguese Atlantic. Seabird indications of mercury contamination in the study region were also assembled with similar information for other regions in a brief meta-analysis for the north Atlantic and adjoining regions.

Fig. 11.1.1
Map of the Portuguese Atlantic showing the main study colonies.



11.1.2. MATERIALS AND METHODS

11.1.2.1. Experimental design

The study region (Fig. 11.1.1) comprises a large oceanic sub-tropical sector (the three Azorean group of islands, Madeira and Salvages) along with a temperate sector off the south-west mainland Portuguese coast (Berlengas, Sado, Sines and Sagres).

Species and monitoring units were selected to ensure varied levels of ecological, spatial and temporal integration within the marine ecosystem: 1) Species with varied feeding ecology reflecting mercury availability in epipelagic (<200 m) and mesopelagic (200-1000 m) food webs (cf. Table 11.1.1, Chapter 8); 2) Chick plumage (down and breast feathers) and eggs reflecting short term exposure in the breeding grounds (c.f. Becker *et al.* 1993a); 3) Adult breast feathers reflecting long-term mercury uptake over the annual cycle (Monteiro & Furness 1995).

11.1.2.2. Sample collection, preparation and analysis

Species and sites sampled were as follows: Bulwer's petrel from eastern Azores (Sta. Maria), Madeira (Deserta Grande) and Salvages (Selvagem Grande); Cory's

TABLE 11.1.1. - Seabird species from the Portuguese Atlantic used in this study and some of their ecological features (sources: Sub-chapters 2.2 and 8.2; Cramp 1977, 1983, 1985; Granadeiro, unpubl. data). ? refers to features poorly known or unknown.

Species	Origin of predominant prey*	Foraging area and range (km) while breeding	Months in breeding area/year	Post-breeding dispersal
Bulwer's petrel *Bulweria bulwerii*	mesopelagic	offshore/ hundreds	6	southwest Atlantic?
Cory's shearwater *Calonectris diomedea*	epipelagic and epi-mesopelagic	offshore/ hundreds	8	north and south western Atlantic
Little shearwater *Puffinus assimilis*	epipelagic	offshore/ hundreds	12	some hundred km?
Madeiran storm petrel *Oceanodroma castro*	mesopelagic	offshore/ hundreds	6	western Atlantic?
Yellow-legged gull *Larus cachinnans*	epipelagic	inshore/ tens	12	from null to hundreds km
Common tern *Sterna hirundo*	epipelagic	inshore/ tens	6	west Africa to Gulf of Guinea
Roseate Tern *Sterna dougallii*	epipelagic	inshore/ tens	6	west Africa to Gulf of Guinea

*Percentage of occurrence in diet over 75%.

shearwater from western (Corvo), central (Graciosa) and eastern (Sta. Maria) Azores, Madeira (Deserta Grande), Salvages (Selvagem Grande) and Berlengas (Berlenga Grande); Little shearwater from eastern (Sta. Maria) Azores and Salvages (Selvagem Grande); Madeiran storm petrel from central (Graciosa) and eastern (Sta. Maria) Azores, Madeira (Deserta Grande), Salvages (Selvagem Grande) and Berlengas (Farilhão Grande); Yellow-legged gull from central (Graciosa, S. Jorge) Azores, Madeira (Deserta Grande and Ilhéu Chão), Salvages (Selvagem Pequena), Berlengas (Berlenga Grande), Sado (estuary), Sines (Ilha do Pessegueiro) and Sagres (Martinhal); Common tern from western (Flores), central (Graciosa, Faial) and eastern (Sta. Maria) Azores; Roseate tern from western (Flores) and eastern (Sta. Maria) Azores. Sampling years ranged from 1993 to 1995, except for some adult samples from Salvages and some tern samples obtained in 1992.

Contour feathers are the most representative for estimating whole-bird mercury content (Furness *et al.* 1986) and 6-10 contour feathers (breast from

adults, ventral from chicks), and down from younger chicks, were plucked from each individual. Species, locality, date, age class (only for plumage) and wing length (only for chick plumage) were recorded for each sample. Other sampling procedures, tissue preparation and storage, total mercury analysis and analytical quality control followed procedures described in Chapter 4. Hereafter mercury refers to total mercury and, unless otherwise stated, values are given in microgram per gram on a fresh weight basis for plumage (ppm, fw) and on a dry weight basis (ppm, dw) for eggs.

11.1.2.3. Data analysis

Potential age-related variations of mercury concentrations in chick plumage must be accounted for in monitoring studies to avoid spurious results (Monteiro & Furness 1995, Chapter 7). Here such a confounding effect was accounted for each species in one of two ways, depending on the structure and size of the available data set. The use of narrow extreme age ranges (e.g. hatchlings or pre-fledglings), where mercury concentrations are normally independent of age (Chapter 7), was preferred for inter-location comparisons. When mercury data showed age-dependency, comparisons were undertaken by analysis of covariance with age (expressed as wing length) as covariate.

Data analysis followed general procedures described in Chapter 4. Parametric tests were preferred and in some cases were performed on logarithmic transformed data to achieve requirements of normality and homocedasticity. One-way analysis of variance or covariance was followed by Tukey-tests for *a posteriori* pairwise comparison of means.

11.1.3. RESULTS

11.1.3.1. Eggs

Mercury concentrations in eggs of the monitor species sampled at various locations in the Portuguese Atlantic are presented in Table 11.1.2. Concentrations in eggs of Bulwer's petrel, which specialises on mesopelagic prey, were several-fold higher than in the remaining species, that feed predominantly on epipelagic prey.

TABLE 11.1.2. - Comparison of mercury concentrations (µg/g dw) in whole egg contents of seabirds from several localities in the Portuguese Atlantic. Values are arithmetic means ±S.D. and sample size bracketed. Within each species, locations with different upper case superscripts (A-C) are significantly different.

Location	Bulwer's petrel	Cory's shearwater	Yellow-legged gull	Common tern	Roseate tern
Western Azores				1.1 ±0.2 (6)^{A,B}	0.6 ±0.2 (9)
Central Azores			1.0 ±0.4 (21)^{A,2}	1.5 ±0.5 (33)^{B,3}	
Eastern Azores	5.9 ±2.4 (16)	1.9 ±0.4 (23)		1.0 ±0.5 (20)^{A}	1.4 ±0.6 (17)
Madeira	5.5 ±1.6 (17)		1.1 ±0.4 (13)^{A,B,2}		
Salvages		2.1 ±1.0 (20)[1]			
Berlengas			1.4 ±0.4 (18)^{A,B,C}		
Sado			1.6 ±0.4 (6)^{B,C}		
Sines			1.6 ±0.4 (29)^{C}		
Sagres			1.4 ±0.3 (19)^{B,C}		
	t-test $t_{31}=0.50$ $P=0.62$		ANOVA $F_{5,100}=7.40$ $P<0.0001$	ANOVA $F_{2,56}=5.61$ $P<0.01$	Mann-Whitney $Z=3.10$ $P<0.005$

[1] Data from Renzoni *et al.* 1986, given for comparison.

[2] One outlier excluded for central Azores (2.9 µg/g dw) and two outliers excluded for Madeira (3.8 and 4.2 µg/g dw).

[3] Inter-year differences in the period 1992-94 were highly significant (see Results).

Egg concentrations in Bulwer's petrel did not differ significantly between Azores and Madeira, and concentrations in Cory's shearwater eggs were identical in Azores and Salvages. Concentrations in Yellow-legged gull eggs differed significantly among locations and showed a tendency for higher values in the mainland coast, especially on Sado and Sines. Within the Azores, concentrations in Common and Roseate tern eggs differed significantly between groups. However, inter-location variation of mercury concentrations in Common tern eggs is comparable to the highly significant (1-way ANOVA, $F_{2,30}=16.17$, $P<0.0001$) inter-year variation observed within central Azores: means ($\pm$S.D, sample size bracketed) in 1992, 1993 and 1994 were, respectively, 1.3 ± 0.3 (15), 1.9 ± 0.5(14) and 0.8 ± 0.2 (4).

11.1.3.2. Chick plumage

Mercury concentrations in down and ventral contour feather of chicks of the monitor species from various locations in the study region are presented, respectively, in Tables 11.1.3. and 11.1.4. Concentrations in chick down show the trend already observed in eggs for higher mercury burdens in species specialised on mesopelagic prey (8.8-19.1 μg/g) comparatively to species which feed predominantly on epipelagic prey (1.8-5.8 μg/g).

Inter-location comparisons showed slight but significant differences of concentrations in down of Bulwer's petrel chicks between the Azores and Salvages, and in down of small Madeiran storm petrel chicks between seasonal populations in central and eastern Azores. Concentrations in down of small Cory's shearwater chicks were uniform throughout the whole study region (cf. Table 11.1.3), but concentrations in ventral feathers were significantly higher in Berlengas than in eastern Azores (c.f. Table 11.1.4). Concentrations in down of Yellow-legged gull chicks were significantly higher in Madeira and Berlengas comparatively to the Azores (c.f. Table 11.1.3), but concentrations in ventral feathers were identical in these three locations (cf. Table 11.1.4); the relatively high concentration in down observed in Sado estuary is noteworthy. Within the Azores, concentrations in the plumage of Common and Roseate tern chicks differed significantly between groups and showed a tendency for consistently lower values in the western group.

TABLE 11.1.3. - Comparison of mercury concentrations (µg/g fw) in down of seabird chicks from several localities in the Portuguese Atlantic. Values are arithmetic means ±S.D., sample size bracketed; within each species, locations with different upper case superscripts (A-B) are significantly different. Codes below species refer to procellariiforms' type of down (primary, D1; secondary, D2) and age range (expressed as wing length, WL in mm) employed.

Location	Species (type of down, age range)					
	Bulwer's petrel (D1,D2; WL<190)	Cory's shearwater (D1, WL<100)	Madeiran storm petrel[1] (D1, WL<40)	Yellow-legged gull (WL<230)	Common tern (WL<30)	Roseate tern (WL<30)
Western Azores		5.8 ±1.2 (25)			2.3 ±0.6 (15)^{A}	1.8 ±0.3 (6)
Central Azores		5.5 ±1.9 (25)	11.6 ±4.0 (8)^{A}	2.1 ±0.8 (36)^{A}	4.4 ±1.9 (5)^{B}	
Eastern Azores	19.1 ±5.1 (26)	5.0 ±1.0 (58)	8.8 ±3.4 (16)^{B}		4.9 ±1.7 (6)^{B}	3.2 ±0.5 (11)
Madeira				3.0 ±1.0 (17)^{B}		
Salvages	15.2 ±2.7 (18)	5.0 ±1.0 (16)				
Berlengas		5.7 ±1.7 (16)	10.1 ±2.8 (14)^{B}	3.6 ±0.9 (15)[3]		
Sado				5.5 ±1.7 (3)[2]		
	ANCOVA $F_{1,41}=4.59$ $P<0.05$	ANOVA $F_{4,135}=1.67$ $P=0.16$	ANCOVA $F_{2,34}=8.25$ $P<0.005$	ANOVA $F_{2,65}=21.66$ $P<0.0001$	ANOVA $F_{2,23}=13.24$ $P<0.0005$	t-test $t_{15}=5.94$ $P<0.0001$

[1] Chicks from different seasonal populations (c.f. Chapter 5): Central Azores, hot season; Eastern Azores and Berlengas, cool season.
[2] Given for comparison but excluded from analysis.

TABLE 11.1.4. - Comparison of mercury concentrations (µg/g fw) in ventral feathers of seabird chicks from several localities in the Portuguese Atlantic. Values are arithmetic means ±S.D., sample size bracketed. Code below species refers to age range employed, expressed as wing length (WL) in mm.

Location	Cory's shearwater (WL>300)	Yellow-legged gull (WL=100-400)	Common tern (WL>140)	Roseate tern (WL>140)
Western Azores			1.1 ±0.4 (10)	0.8 ±0.2 (19)
Central Azores		2.3 ±1.0 (34)	1.5 ±0.4 (19)	
Eastern Azores	0.7 ±0.2 (30)		1.4 ±0.4 (32)	1.1 ±0.2 (14)
Madeira		2.6 ±0.8 (22)		
Salvages				
Berlengas	1.1 ±0.3 (25)	2.4 ±0.5 (28)		
	t-test $t_{53}=6.85$ $P<0.0001$	ANOVA $F_{2,88}=0.19$ $P=0.83$	ANOVA $F_{2,58}=3.08$ $P=0.053$	t-test $t_{31}=4.14$ $P<0.0005$

11.1.3.3. ADULT PLUMAGE

Mercury concentrations in adult breast feathers of monitor species from several localities in the study region are presented in Table 11.1.5. As for eggs and chick plumage, concentrations in adult breast feathers were many-fold higher in the petrels (11.1-23.9 µg/g) than in shearwaters and the gull (2.7-5.8 µg/g). Concentrations in seasonal populations of Madeiran storm petrel differed significantly in central Azores ($t_{125}=7.86$, $P<0.0001$) but not in Madeira ($t_{28}=1.37$, $P=0.18$).

Concentrations in adult breast feathers did not differ significantly between locations for Bulwer's petrel and Cory's and Little shearwaters. Significant inter-location differences were found in both seasonal populations of Madeiran storm petrel and in Yellow-legged gull.

11.1.4. DISCUSSION

11.1.4.1. VALIDITY AND ASSUMPTIONS

Several validity aspects and assumptions must be considered if mercury concentrations in seabird tissues are to reflect variations in environmental contamination with mercury. The use of seabirds as monitors of mercury relies on experimental and field evidence showing that mercury levels in their tissues reflect levels in diets (Lewis & Furness 1991, Sub-chapter 8.2, Chapters 9 and 10), which in turn reflect ecosystem contamination (Renzoni *et al.* 1986, Chapter 8). Nevertheless, interpreting mercury burdens in seabird tissues for monitoring purposes requires a good deal of knowledge on unwanted intra-specific sources of variation (assessed in Chapter 7) and on aspects of the ecology of each particular monitor species (summary in Table 11.1.1) that are examined below.

1. The dietary composition of a particular seabird species must remain constant over the study region. Its diet should be well known and should show no systematic variation between sampling locations. Species with relatively narrow diets, like the petrels and shearwaters, have an enhanced monitoring value comparatively to species with more generalist diets, like the Yellow-legged gull and to some extent the terns.

TABLE 11.1.5. - Comparison of mercury concentrations (µg/g fw) in breast feathers of adult seabirds from several localities in the Portuguese Atlantic. Values are arithmetic means ±S.D., sample size bracketed; within each species, locations with different upper case superscripts (A-B) are significantly different.

Location	Bulwer's petrel	Cory's shearwater	Little shearwater	Hot season Madeiran storm petrel[1]	Cool season Madeiran storm petrel[1]	Yellow-legged gull
Western Azores		5.8 ±1.3 (30)				
Central Azores		5.0 ±1.3 (19)		11.1 ±3.4 (107)^{A}	17.7 ±3.9 (20)^{A}	5.5 ±1.0 (26)^{A}
Eastern Azores	22.6 ±4.1 (66)	5.3 ±1.8 (263)	3.1 ±1.0 (79)		18.3 ±4.8 (83)^{A}	
Madeira	23.9 ±5.2 (15)	4.7 ±1.2 (15)		13.7 ±2.6 (15)^{B}	15.3 ±3.4 (15)^{A,B}	4.2 ±1.3 (7)^{A,B}
Salvages	20.8 ±5.2 (40)	5.2 ±1.6 (41)	2.7 ±0.8 (19)	15.6 ±3.8 (17)^{B}		5.4 ±2.0 (16)^{A,B,2}
Berlengas		5.3 ±1.5 (24)			14.3 ±3.7 (31)^{B}	4.4 ±1.4 (26)^{B}
	ANOVA $F_{2,118}=3.03$ $P=0.052$	ANOVA $F_{5,386}=1.02$ $P=0.40$	t-test $t_{96}=1.80$ $P=0.075$	ANOVA $F_{2,136}=15.31$ $P<0.0001$	ANOVA $F_{3,145}=7.35$ $P<0.0005$	ANOVA $F_{3,71}=3.63$ $P<0.05$

[1] Allocation of birds to seasonal populations followed Chapter 5 for birds sampled in the Azores and it was based on month(s) of sampling for other locations: Madeira, hot season in June and cool season in November; Salvages, hot season in July-early August; Berlengas in September-October.

[2] One outlier excluded (13.3 µg/g fw).

2. Different seabird species exhibit varied dietary specialisations and tend to feed at varying distances from land (cf. Table 11.1.1). Therefore, it is possible to select monitor species to make inferences about relative environmental variations of mercury loads in different food chains, either in epipelagic or mesopelagic environments, and in inshore or offshore waters.

3. Seasonal movements of seabirds and their consequent exposure to mercury in different localities (c.f. Table 11.1.1) may lead to varying levels of spatial representativeness. Mercury levels in adult feathers (sampled in breeding areas) of migratory species may reflect both exposures to mercury in breeding and non-breeding areas. However, mercury intake during the breeding period seems to be largely responsible for mercury concentrations in contour feathers and other tissues of seabirds with long breeding seasons (Monteiro *et al.* 1995, Chapter 7). Mercury intake in non-breeding areas may play some role in determining levels in feathers of species with short breeding seasons like terns (Burger *et al.* 1992a), although there is contrary evidence (Thompson *et al.* 1993).

4. Mercury concentrations in eggs and chick plumage reflect environmental levels of mercury from a clearly defined time period and limited parental foraging range at the breeding areas (Furness 1993, Monteiro & Furness 1995, Chapter 9).

11.1.4.1. Spatial patterns

There are few published data on diets, breeding and post breeding dispersion of seabirds in the study region (reviews in Sub-chapters 2.2 and 8.2; summary in Table 11.1.1) and this makes difficult a full explanation of the observed inter-location differences in mercury concentrations. Literature and ongoing studies suggest that diets of procellariiforms (petrels and shearwaters) are similar among locations, including the partitioning of epipelagic and mesopelagic resources between, respectively, shearwaters and petrels (Zonfrillo 1986, Sub-chapter 8.2, Granadeiro pers. com.). Thus, geographical differences of mercury concentrations potentially indicated by procellariiforms would reflect primarily variations in the environmental availability of mercury in their food webs. The situation with the larids (gull and terns) is not that straightforward. Potential geographical variations in mercury contamination indicated by the generalist and opportunistic feeding Yellow-legged gull should take into account possible dietary variations throughout the study region. Given that adults from the mainland colonies (Berlengas to Sagres) are thought to feed to some extent on refuse at rubbish tips (Granadeiro

pers. com.), they are expected to exhibit lower mercury concentrations in tissues than adults from colonies in the three Atlantic archipelagos, which feed mainly on marine resources (Sub-chapter 2.2, Leonzio *et al.* 1986); this may be true also for large chicks, since parental food provisioning by the allied Herring gull *Larus argentatus* in colonies near rubbish tips shows important shifts from fish to meat with increasing chick age (Nogales *et al.* 1995). Some Yellow-legged gull adults specialise on preying upon other seabirds (Sub-chapter 2.2) and that may account for abnormal high mercury concentrations in their tissues (e.g. the outliers in Tables 11.1.2. and 11.1.5). Gull and terns diet at some Azores colonies include in some seasons up to 10-20% of mesopelagic fish (Hamer *et al.* 1994; Granadeiro, Monteiro, Ramos unpubl. data). Given the enhanced mercury burdens in mesopelagic fish (Sub-chapter 8.1) it is crucial to make also some allowance for that potential confounding effect when inferring geographical variations in mercury contamination from concentrations in their tissues.

The results (Tables 11.1.2 to 11.1.5) show that fourteen out of twenty inter-location comparisons of mercury concentrations in eggs, chick plumage or adult breast feathers yield statistically significant differences. Six of those significant differences arise from microgeographic variations of mercury concentrations in Common and Roseate terns between western and central or eastern Azores and are not thought to reflect genuine variation in mercury contamination. Indeed, the tendency for lower mercury concentrations in tern eggs and plumage of chicks in the western group may be fully accounted by differences in the relative importance of mesopelagic prey as indicated by the following crude mass balance. Based on an average hourly consumption rate of 1.0 fish over 14 hours/day (unpubl. data) and typical mercury concentrations in epipelagic and mesopelagic prey fish of, respectively, 60 and 300 ng/g dw (Sub-chapter 8.1) the total mercury intake of a tern chick at a given age will be 40% higher in a diet comprising 10% of mesopelagic fish comparatively to a diet comprising only epipelagic fish. Furthermore, the microgeographic differences under discussion are in the same order of magnitude of inter-year variations in Common tern egg mercury concentrations in central Azores (see results). Five other significant differences are slight variations of chick and adult plumage concentrations in seasonal populations of Madeiran storm petrel, down of Bulwer's petrel chicks and feathers of adult Yellow-legged gull, which did not rise above the intra-specific variability quantified in Chapter 7 and are, therefore, attributable to 'background noise'. Thus, only the remaining three significant differences are thought to reflect

genuine environmental variation in mercury contamination. Samples of Yellow-legged gull eggs and chick down and ventral feathers of Cory's shearwater chicks indicate a tendency for higher availability of mercury in the mainland coastal sector, with a slight peak at Sado and Sines. The increases of mercury concentrations in samples of Yellow-legged gull are particularly noteworthy, given expected lower concentrations due to prevalence of feeding on refuse. Given that the extent of feeding on discards and offal at the mainland locations is unknown it is impossible to make allowance for this potential confounding factor.

Overall, there is a general agreement in the information provided by species used as monitors of epipelagic and mesopelagic compartments and by different types of monitoring units employed. They indicate a many-fold difference in mercury contamination between vertical compartments coupled with an even distribution of contamination in both compartments across most of the study region, from the remote mid-north Atlantic to coastal south-west Europe. There is also evidence of an apparent slightly enhanced mercury contamination in the epipelagic food web adjacent to the south west Portuguese coast, which requires further information for a confident elucidation.

The environmental significance of the above results must be perceived within the current framework for mercury biogeochemical cycling (EPMAP 1994): the level of contamination at any location of the terrestrial or marine environment is composed of contributions from the global cycle, and regional and local sources. The relatively uniform distribution of mercury contamination observed in the oceanic environment on a regional basis, suggests, as major source, atmospheric deposition of elemental mercury associated with the global cycle. Microgeographic patterns found at the mainland coast correlate with patterns of increasing human activity and may be due to atmospheric deposition of particulate mercury emitted from local-scale sources such as power plants burning coal and fuel (Sines and Sado), oil refining and other industries (Tagus and Sado estuaries, Sines). Potential sources of mercury at a regional scale are volcanism and the presence of Mediterranean water, and they have been invoked to explain increased concentrations of mercury in near-bottom water (3,200 m) of a mid-Atlantic rift valley (Carr *et al.* 1974). Superficial volcanism and geothermal activity constitute important local sources of atmospheric mercury in the extremely active Icelandic area (Siegel *et al.* 1973, Olafson 1975) but the influence of erupting volcanoes is highly transient and has a short-term impact on superficial seawater mercury concentrations (Olafson 1975). Given the absence of important eruptions in the

last decades in the Portuguese Atlantic and the lack of regional differences in mercury contamination between comparatively more active (central and eastern Azores) and less active (Madeira and Salvages) volcanic areas in this study, it appears that volcanism plays an unimportant contribution to seabird indications of mercury contamination presented in this study. The same seems true for the possible influence of mercury-enriched Mediterranean water (UNEP/FAO/WHO 1987) which flows into the north Atlantic at depths below 1000 m (Kennett 1982), i.e. in the bathypelagic compartment that is not integrated by seabird species here employed as monitors.

11.1.4.2. North Atlantic meta-analysis

Table 11.1.6 presents mercury concentrations in species and/or monitoring units that reflect mercury contamination of local (presumably epipelagic) food webs from a variety of areas in the temperate and sub-tropical north Atlantic, adjoining seas and inland north-east America. An overall comparison of these seabird indications of environmental availability of mercury reveals three main aspects. First, the contamination levels in the Portuguese Atlantic tend to match the lowest values of ranges given and thus are in general agreement with levels reported for populations of the same species elsewhere in 'non-polluted' areas. Second, the analysis confirms local and regional hot-spots at the Mediterranean (UNEP/FAO/WHO 1987) and certain locations of the German coast (e.g. Becker *et al.* 1993a,b; Slemr & Langer 1992). Third, excluding these hot-spots, the overall level of contamination does not appear to differ much between coastal and oceanic environments as claimed by Davis (1993). This meta-analysis should be taken as indicative and some allowance must be made for the potential lack of consistency in diets of particular species across the whole north Atlantic, although trophic levels presumably tend to remain unchanged. There is some evidence that mercury contamination may be relatively uniform within the north Atlantic at a given trophic level in regionally distinct epipelagic food webs. This is suggested by the close agreement between ranges of mercury concentrations in adult feathers (1.0 to 6 μg/g) from more than twelve seabird species which feed predominantly (or exclusively) on distinct epipelagic fish assemblages from the sub-tropical (Monteiro *et al.* 1995) to temperate and boreal (Thompson *et al.* 1992) north-east Atlantic.

TABLE 11.1.6. - Mercury concentrations in whole egg contents (µg/g dw[1]) and plumage (µg/g fw) of chicks (down, contour feathers) or adults (contour feathers) from selected seabirds from a variety of areas in the North Atlantic, adjoining seas and inland north-east America.

Species	Monitoring unit	Area	Mean	Source
Cory's shearwater	egg	Mediterranean	4.8-7.3	Renzoni *et al.* 1986
		Azores/Salvages, NE Atlantic	1.9-2.1	Renzoni *et al.* 1986, This study
Gull[2]	egg	N. coast of Germany	0.4-5.6	Becker *et al.* 1993a
		Ontario/Great Lakes, W. Canada	1.0-1.5	Ryder 1974[3], Gilman *et al.* 1977[3]
		Norway	0.2-0.8	Fimreite *et al.* 1974[3], Barrett *et al.* 1985[3]
		Mediterranean	1.9-3.5	Lambertini 1982, Focardi *et al.* 1986
		Azores/Madeira, NE Atlantic	1.0-1.1	This study
		SW Iberian coast, Portugal	1.4-1.6	This study
	down	N. coast of Germany	1.4-10	Becker *et al.* 1993a, 1994
		Mediterranean	8.8	Lambertini 1982
		Azores/Madeira, NE Atlantic	2.1-3.0	This study
		SW Iberian coast, Portugal	3.0-5.5	This study
	chick feathers	N. coast of Germany	1.2-11	Becker *et al.* 1993
		Portuguese Atlantic	2.3-2.6	This study
	adult feathers	Wadden Sea, Germany	5.3	Lewis *et al.* 1993
		Isle of May, Scotland	3.4	Thompson *et al.* 1990
		Portuguese Atlantic	4.2-5.5	This study

(cont.)

TABLE 11.1.6. - (cont.)

Species	Monitoring unit	Area	Mean	Source
Common tern	egg	Jade Bay, Germany	1.8	Becker *et al.* 1993c
		Elbe estuary, Germany	6-7	Becker *et al.* 1993b
		Cedar Beach/Barnegat Bay, E. USA	1.4	Gochfeld & Burger 1987
		Azores, NE Atlantic	1.0-1.5	This study
	down	Jade Bay, Germany	5.9	Becker *et al.* 1994
		Elbe estuary, Germany	33	Becker *et al.* 1993a
		Azores, NE Atlantic	2.3-4.9	This study
	chick feathers	Jade Bay, Germany	3.1	Becker *et al.* 1994
		Scotland/Shetland	1.4-1.8	Furness *et al.* 1994
		Long Is./Bird Is., E. USA	1.4-3.0	Gochfeld 1980, Burger *et al.* 1994
		Azores, NE Atlantic	1.1-1.5	This study
Roseate tern	egg	Eastern USA	0.8-1.0	Burger & Gochfeld 1993
		Azores, NE Atlantic	0.6-1.4	This study
	chick feathers	Eastern USA	1.2-2.0	Burger & Gochfeld 1993
		Azores, NE Atlantic	0.8-1.1	This study

[1] Concentrations expressed in fresh weight basis were converted to dry weight values by assuming a moisture of 75% (Chapter 4).
[2] Yellow-legged gull (Portuguese Atlantic, Mediterranean) or Herring gull (other locations) from *argentatus/cachinnans* complex (Cramp 1983).
[3] Quoted by Lewis *et al.* 1993

Mercury concentrations in other marine biota from the Azores (Monteiro & Lopes 1990, Monteiro *et al.* 1991, Monteiro *et al.* 1992) and recent mercury concentrations in seawater from the north Atlantic (Slemr *et al.* 1981) are in agreement with the above meta-analysis for the north Atlantic. In addition, studies of the latitudinal distribution of mercury in surface waters and atmosphere over the Atlantic from 42°N to 32°S (Slemr *et al.* 1981, Slemr & Langer 1992) show an interhemispherical difference, with concentrations in the northern hemisphere about 50% higher than in the southern hemisphere. Altogether, the apparent meso-scale background uniformity of mercury contamination in the sub-tropical and temperate north Atlantic provides further evidence of predicted global pollution by mercury due to atmospheric deposition at long distance from emission sources.

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Sub-chapter 11.2

HISTORICAL INCREASES IN EPIPELAGIC AND MESOPELAGIC ENVIRONMENTS

11.2.1. INTRODUCTION

The global cycle of mercury is dominated by anthropogenic and natural emissions of gaseous mercury (Hg^0) to the atmosphere and atmospheric transport of Hg^0 is the main pathway for the distribution of mercury at the Earth's surface (EPMAP 1994, Mason *et al.* 1994a). Air-sea exchange processes, as oceanic production with subsequent evasion of Hg^0 and atmospheric deposition are a crucial part of the global biogeochemical cycle of mercury (Fitzgerald 1989, EPMAP 1994). Mass balances and model-based analysis indicate that human activities are responsible for 50 to 75% of the total current atmospheric emissions (EPMAP 1994, Mason *et al.* 1994a, Hudson *et al.* 1995). That influence is prolonged by recycling of mercury between the surface oceans and the atmosphere and a major proportion of contemporary oceanic effluxes are a re-emission of deposited anthropogenic mercury (Mason *et al.* 1994a, Hudson *et al.* 1995). If the anthropogenic emissions ceased today the anthropogenic load would not be eliminated for fifteen-twenty years (EPMAP 1994, Mason *et al.* 1994a).

The impact of anthropogenic mercury emissions (chiefly from fossil fuel combustion, waste incineration, smelting; Nriagu & Pacyna 1988) has been assessed using historical records in indicator media. Lake sediments and peat bogs were used successfully to quantify historical trends in terrestrial atmospheric deposition (e.g. Pheiffer-Madsen 1981, Rada *et al.* 1989). Top predator tissues (fish muscle, fur and feathers) were used to assess the impacts of anthropogenic inputs on mercury bioavailability and hazards in aquatic ecosystems but most indications are hampered by methodological uncertainties (Furness *et al.* 1990). Ice-cores from remote regions have not provided reliable data on changes because of sampling and analytical limitations (Wolff 1990, Vandal et al. 1993). Nonetheless, most records of historical change were affected by regional inputs (Pheiffer-Madsen 1981, Appelquist *et al.* 1985, Nater & Grigal 1992, Swain *et al.* 1992, Thompson *et al.* 1993a, Hermanson 1993, Benoit *et al.* 1994) and should not be invoked to infer global-scale changes or for extrapolations to pristine areas. Thus, empirical evidence of global impact of anthropogenic emissions is still scanty (Fitzgerald 1995). It is a current critical research need to broaden the spatial and ecological representativeness of historical records and assess trends in deposition at pristine oceanic sites (EPMAP 1994). It is particularly crucial to discriminate between marine vertical compartments, given the potential transport of anthropogenic-derived mercury into deep waters (Hudson *et al.* 1995) where

methylation (Mason & Fitzgerald 1990) leads to enhanced bioaccumulation by mesopelagic organisms (Sub-chapter 8.1).

Here, mercury concentrations in feather time-series (1886-1994) from seabirds breeding in the sub-tropical North-east Atlantic were used to infer historical trends in global mercury contamination in the epipelagic and mesopelagic environments due to anthropogenic mercury deposition at long distance from emission sources.

11.2.2. MATERIALS AND METHODS

The study area covers a wide oceanic region from the Azores to Madeira and Salvages (30-40°N, 8-15°W). The monitor species were selected on basis of *a priori* information on the ecology of the local seabird assemblage. Although they all feed predominantly on fish, ecological segregation leads to dietary specialisation on epipelagic or mesopelagic resources (Sub-chapter 2.2), thus ensuring vertical integration of mercury contamination (Sub-chapter 8.2). Species and sites sampled were as follows: epipelagic monitors -Cory's shearwater *Calonectris diomedea borealis* (Azores, Madeira, Salvages), Little shearwater *Puffinus assimilis baroli* (Azores, Salvages), Common tern *Sterna hirundo* (Azores); mesopelagic monitors - Bulwer's petrel *Bulweria bulwerii* (Madeira, Salvages), Madeiran storm petrel *Oceanodroma castro* (Madeira, Salvages).

Contemporary samples (post-1990) were obtained from adult birds at breeding colonies and historical samples (pre-1970) were obtained from dated preserved adult's study skins held in museum collections (listed in the Acknowledgements). Contour feathers are the most representative for estimating whole-bird mercury content (Furness *et al.* 1986) and up to 12 ventral feathers were collected from each individual, by plucking from live birds or cutting with scissors from study skins, and placed in polyethylene bags prior to analysis. Feather samples with major dust-surface contamination, likely to alter feather weight, were subjected to a chloroform/acetone washing regime outlined in Chapter 4. Historical samples were subjected to an initial fractionation to extract only the organic mercury present and overcome potential post-mortem contamination of study skins with inorganic mercury used as preservative (Furness *et al.* 1990). As mercury in seabird feathers has been shown to be entirely organic (Thompson & Furness 1989), this allows a direct comparison with total mercury

concentrations in uncontaminated contemporary feather samples. That was confirmed for the monitor species by replicate determinations of organic and total mercury concentrations (range: 0.9-22.8 μg/g fw) in contemporary samples, which yielded a mean organic fraction of 101.0% (S.D. = 12.9, range = 85.7-122.9, n = 10).

Mercury concentrations of contemporary samples consist of appropriate data sets from Chapter 7 and Sub-chapter 11.1. Data analysis followed procedures described in Chapter 4. Parametric tests were preferred and in some cases logarithmic transformation of data was required to achieve requirements of normality and homoscedasticity.

11.2.3. RESULTS

The numbers of preserved historical specimens available preclude an assessment of historical changes by location. However, mercury concentrations in contemporary feather samples did not differ significantly among locations (Sub-chapter 11.1) and combined mercury concentration data were used in subsequent analysis for each species.

A comparison of mercury concentrations in birds sampled pre-1931 and post-1990 (Table 11.2.1) shows increases in all species. Assuming linearity, overall estimates of the increase rate were 1.1 $\%.yr^{-1}$ in the epipelagic monitors and 3.5 $\%.yr^{-1}$ in the mesopelagic monitors, the rates differing by over three-fold.

The relatively large number of samples available for Cory's shearwater and Bulwer's petrel permitted the fine-time resolution of historical increases illustrated in Fig. 11.2.1 and summarised in Table 11.2.2. For both species, mercury concentrations increased in a highly significant way (1-way ANOVAs: Cory's shearwater, $F_{3,272} = 78.84$, $P < 0.0001$; Bulwer's petrel, $F_{3,81} = 69.47$, $P < 0.0001$) across the four time-periods in Table 11.2.2. It is noteworthy that historical increases occurred as early as 1900-1931 relative to 1885-1900 and at a higher rate than in subsequent time-periods. Whereas the value of this indication of short-term patterns is weakened by some small sample sizes, it is strengthened by the consistency of indications from epipelagic (Cory's shearwater) and mesopelagic (Bulwer's petrel) monitors. Long-term increases indicated by this analysis are higher than those given in Table 11.2.1 for the same species, because those resulted from a comparison of contemporary levels with historically recent 'background' values.

TABLE 11.2.1. - Increases of mercury concentrations (µg/g fw) in contour feathers of adult seabirds from the sub-tropical North-east Atlantic between pre-1931 and post-1990.

Species	Pre-1931[1]	Post-1990	Increase[2] (%)	Increase[3] (%.yr^{-1})	Statistical test[4]
Cory's shearwater	1921 2.7±0.9 (0.6-4.6), 2.7 (48)	1993 5.4±1.6 (1.9-10.6), 5.3 (219)	100	1.4	Z=9.50, P<0.0001
Little shearwater	1895 1.7±0.7 (0.8-3.2), 1.6 (15)	1993 2.8±1.0 (1.8-6.9), 2.4 (34)	65	0.7	t=3.82, P<0.0005
Common tern	1927 1.1±0.5 (0.5-2.1), 1.1 (15)	1992 2.0±0.6 (1.2-3.5), 2.1 (22)	82	1.3	t=4.90, P<0.0001
Bulwer's petrel	1903 6.0±3.9 (0.9-15.1), 5.1 (30)	1993 21.6±5.3 (12.2-33.8), 21.9 (55)	260	2.9	Z=7.39, P<0.0001
Madeiran storm petrel	1896 3.0±0.3 (1.3-6.8), 2.8 (16)	1993 14.9±3.4 (9.3-24.9), 14.3 (47)	397	4.1	Z=5.94, P<0.0001

[1] Values for each time-period are: above, median year; middle, mercury concentrations $\bar{x}\pm$ S.D. (range), median; below, sample size.

[2] Absolute percent increase based on mean mercury concentrations.

[3] Average annual rate of increase equal to absolute percent change divided by range of median years in the two periods.

[4] Z, Mann-Whitney; t, t-test.

Fig. 11.2.1
Historical variation of mercury concentrations in contour feathers of (A) Cory's shearwater and (B) Bulwer's petrel from the sub-tropical North-east Atlantic.

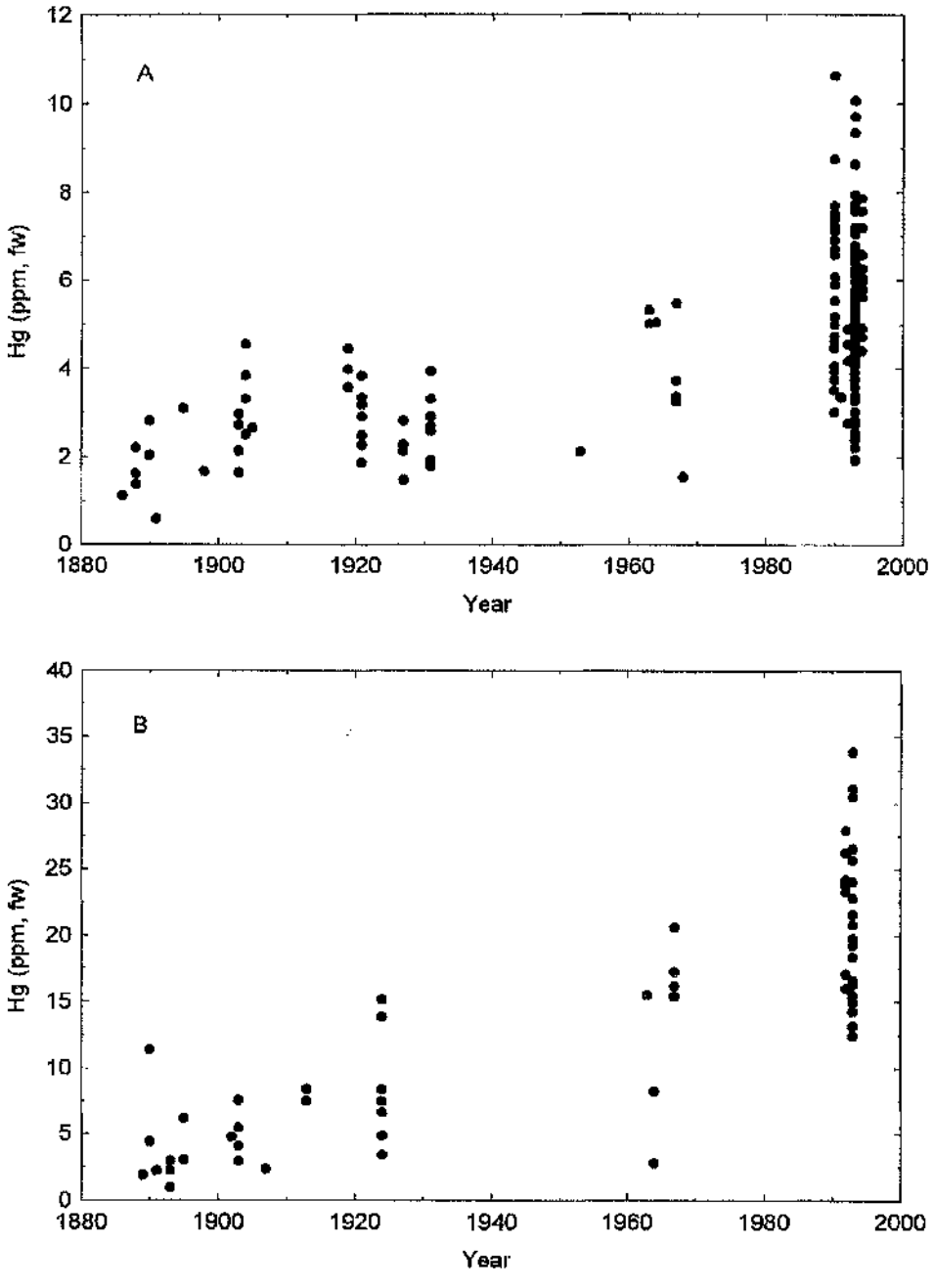

TABLE 11.2.2. - Short-term historical increases of mercury concentrations (µg/g fw) in contour feathers of adult Cory's shearwater and Bulwer's petrel from the sub-tropical North-east Atlantic and long-term increase (%.yr^{-1}) between pre-1900 and post-1990.

Species	1885-1900[1]	1900-31	1950-70	1992-94	Long-term increase
Cory's shearwater	1890 1.8±0.8 (10) –	1921 2.9±0.8 (38) 2.0	1967 3.9±1.4 (9) 0.7	1993 5.4±1.6 (219) 1.5	1.9
Bulwer's petrel	1892 3.7±3.1 (10) –	1919 7.1±3.8 (20) 3.4	1967 14.3±5.1 (10) 2.1	1993 21.6±5.3 (55) 2.0	4.8

[1] Values for each time-period are: above, median year; middle, mercury concentration $\bar{x}\pm$ S.D. (n); below, short-term annual rate of increase relative to previous period, estimated as in Table 11.2.1.

11.2.4. DISCUSSION

11.2.4.1. VALIDITY AND ASSUMPTIONS

The use of seabird feathers to infer historical trends in mercury contamination relies on the following rationale: 1) Seabirds excrete dietary mercury in a linear dose-dependent fashion into the plumage by endogenous incorporation during feather growth (Chapters 9 and 10); 2) Mercury is stably bound to the feather keratin (Appelquist *et al.* 1984) and levels are not affected by atmospheric deposition (Hahn *et al.* 1993). In addition, the accuracy of monitoring programmes depends on knowledge of potential confounding factors (Monteiro & Furness 1995). Historical trends observed here cannot be explained by methodological bias, as they rise well above the baseline 'noise' quantified in *a priori* study of the intra-population contemporary variability of mercury in the monitor species (Chapter 7). However, it is necessary to assume that the diet of monitor species has remained constant over the study period to ascribe the observed trends to genuine environmental change. This seems reasonable regarding the ecology of the monitor species (Sub-chapter 2.2.) and that major changes in ecosystem structure are unlikely given the local low levels of exploitation of pelagic resources until recently (Santos *et al.* 1996). Ecological processes underlying segregation

and partitioning of epipelagic and mesopelagic resources should have remained unchanged. Moreover, indicator species have narrow and relatively inflexible diets (especially petrels and shearwaters), which are unlikely to have suffered historical changes, and trends observed are consistent between species feeding on different prey within the same environment. The spatial representativeness of observed trends may be questioned on grounds of seasonal movements of monitor species and their consequent exposure to mercury in different localities. However, there is a good deal of field and experimental evidence indicating mercury intake during the breeding period to be largely responsible for mercury concentrations in contour feathers of the monitor species employed here (Monteiro *et al.* 1995, Chapter 7, Chapter 9), which remain for six or more months in breeding grounds. At the very least, the trends may be taken as reflecting contamination over the wide Atlantic sector between North and South mid-latitudes, where the migratory species seem to spend their entire annual cycle (Sub-chapter 2.2).

11.2.4.2. TRENDS

The observed long-term increases of mercury contamination near the apex of the epipelagic food web (0.7-1.9%.yr^{-1}) are consistent with current estimates of increase rates in the global background of mercury due to anthropogenic inputs. Mass balances suggest that global mercury concentrations in atmosphere and surface oceans increased three-fold since pre-industrial times (Mason *et al.* 1994a), which translates to an average rate of 1.3%.yr^{-1} over 150 years. Direct measurements over the Atlantic Ocean indicate increases in atmospheric mercury concentrations by 1.5 and 1.2%.yr^{-1} for the Northern and Southern Hemisphere, respectively, for the period 1977-1990 (Slemr & Langer 1992). The proportionality between increases in anthropogenic atmospheric inputs and epipelagic organisms is presumably due to the rapid equilibrium of mercury between the atmosphere and the surface ocean (Fitzgerald 1989, Mason *et al.* 1994b). The trends observed here for the epipelagic oceanic environment are consistent with peat bogs (Benoit *et al.* 1994), lake sediments (Swain *et al.* 1992) and seabird feathers (Thompson *et al.* 1992), all recording two to four-fold increases since the beginning of the 19th century in mid-latitudes of the Northern Hemisphere. Slight increases in the sub-Antarctic region between pre-1950 and post-1950 were also indicated by seabird feathers (Thompson *et al.* 1993b).

The observed long-term increases of mercury contamination near the apex of the mesopelagic food web (2.9-4.8%.yr^{-1}) correspond to a three-fold amplification of the anthropogenic-derived pulse of mercury comparatively to analogous rates for the epipelagic food web, atmosphere and surface ocean (above). Explanations for this difference must be sought in the particular biogeochemistry of mercury in low-oxygen sub-thermocline seawater, which results in microbial mediated methylation of reactive mercury supplied by scavenging of particulate mercury from the mixed-layer (Mason & Fitzgerald 1990, Mason & Fitzgerald 1993). The mesopelagic trend may simply reflect enhanced bioaccumulation at low trophic levels (Sub-chapter 8.2) due to high bioavailability of methylmercury. However, given that methylation is substrate-limited (Mason & Fitzgerald 1993), the mesopelagic magnification of historical superficial increases may also reflect long-term increase in fluxes of particulate mater from the mixed-layer to sub-thermocline waters. There is clearly considerable scope for more research in this topic regarding conflicting views on the role of mixing into the ocean interior as a sink of anthropogenic-derived mercury (Hudson *et al.* 1995 versus Mason *et al.* 1994a).

Historical increases do not appear to have been linear in the epipelagic or in mesopelagic environments, mainly due to enhanced increases at the turn of the century. This pattern is remarkably consistent with variations in rates of atmospheric mercury deposition in mid-continental North America in the same period (Benoit *et al.* 1994). Such coincidence may be accounted for by the simultaneous concurrent peak use and loss of mercury to the atmosphere from gold and silver mines in the period 1850-1900, thought to have exerted a dominant and long-lived influence on the global mercury cycle (Nriagu 1994). Apparent declines in atmospheric mercury deposition post-1960 recorded in mid-continental North American peat bog, presumably reflect regional-scale changes in emissions (Benoit *et al.* 1994) and were not recorded here in seabird feathers.

11.2.4.3. Environmental implications

The trends presented above must be perceived under the extreme role of atmospheric processes on mercury environmental cycling. The flux from the atmosphere at any one location on the Earth's surface is composed of contributions from the natural global cycle, the perturbed global cycle, and regional and local sources (EPMAP 1994). If the only significant mode of deposition of

atmospheric mercury is associated with the global cycle, then mercury distribution in oceanic environments should be relatively uniform on a regional basis, whereas if mercury deposition is dominated by regional or local processes, spatial patterns in mercury distribution would emerge. Evidence of the predominant role of global-scale processes in the study area came from a companion study (Sub-chapter 11.1) showing a contemporary uniform distribution of mercury contamination in the epipelagic and mesopelagic environments. Assuming that main regional-scale mercury-related process (fluxes from volcanism and occurrence of Mediterranean water below 1000m) were steady over the study period, the observed historical increases reflect the impact of the perturbed global cycle of mercury in the sub-tropical North-east Atlantic. Moreover, a similar impact of the perturbed global cycle in mid-latitude terrestrial and marine ecosystems is suggested by consistent increase rates in the North Atlantic (Slemr & Langer 1992, this study) and remote mid-continental North America (Swain *et al.* 1992, Benoit *et al.* 1994).

The observed increases in mercury concentrations in seabirds feeding near the apex of food chains provide an empirical linkage between increasing accumulation of methylmercury in aquatic organisms and anthropogenic influence in the global mercury cycle. Large increases, if substantiated further, especially in mesopelagic organisms, are of concern because of the current public-health problem resulting from widespread incidence of elevated levels of methylmercury in fish (WHO 1990, Fitzgerald & Clarkson 1991). Besides, potential interactions of environmental change on the global mercury cycle should not be neglected. Upwelling intensification due to climate change will tend to enhance primary productivity (Bakun 1990) and thus may increase both oceanic evasion of elemental mercury and scavenging of particulate matter to sub-thermocline waters for subsequent methylation. Changes in the general chemistry of the atmosphere, as increases in oxidants and particles can, somehow, affect residence times for mercury in the atmosphere and alter atmospheric deposition rates (Hudson *et al.* 1995). The uncertainties concerning the outcome of anthropogenic-derived global processes in current levels of mercury contamination beg for an appraisal of long-term trends at remote locations. In this respect, and given that mercury cycling in aquatic ecosystems includes many biologically-mediated processes (Beijer & Jernelov 1979, Mason & Fitzgerald 1993), seabird study skins present good prospects for a comprehensive appraisal of the ecological hazards of global pollution by mercury into epipelagic and mesopelagic food webs.

11.2.5. REFERENCES

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CHAPTER 12

GENERAL DISCUSSION

The oceans play a pre-eminent role in the global mercury cycle. Given that the behaviour of mercury in marine ecosystems is dominated by biologically-mediated processes, biomonitors are advantageous to assess mercury bioavailability. Top predators in particular will better reflect mercury hazards to the ecosystem and Humans, as they integrate mercury contamination over food webs. In this respect, seabirds offer varied levels of ecological, spatial and temporal integration and have been successfully used as cost effective monitors of mercury contamination in the marine environment, as discussed in Chapter 3.

The use of seabirds as monitors is not entirely straightforward and requires a good knowledge of the ecology of monitor species and of baseline 'noise' from potential confounding factors. Thus, this study started with an appraisal of the structure and functioning of the Azores seabird assemblage in Chapter 2, which provided simultaneously a contribution to the understanding of the ecology of seabird populations in the sub-tropical portion of the North Atlantic. It provided evidence of a strong impact of human activities on the seabird community in the Azores, following colonisation in the 15th century. That resulted in dramatic declines for most species, that now breed on small islets. Ecological segregation in time of breeding and use of food resources is evident among the Procellariiformes. While the petrels are specialised in exploiting vertically migrating mesopelagic fish, the shearwaters feed preferentially on a wide variety of shoaling epipelagic fish and squid.

The study of the seabird assemblage led to the discovery of temporally segregated populations of the Madeiran storm petrel *Oceanodroma castro* in the Azores, breeding sympatrically on some islets, as described in Chapter 5. Phenotypic differentiation between the hot season and cool season populations in adults, eggs and chicks was interpreted as an adaptative response to different environmental conditions in the two seasons. These populations may represent a rare case of sympatric speciation through temporal partitioning of reproduction and a future measure of the extent of divergence using DNA techniques may show that they would be better treated as sibling taxa.

Accordingly to current conceptual models, moult is a key factor ruling mercury dynamics in seabirds. Therefore, its knowledge is essential when interpreting mercury concentrations in seabird tissues. The study of moult phenology within the Azores seabird assemblage revealed a considerable overlap of primary moult and breeding season in Cory's Shearwater *Calonectris diomedea borealis* breeders, which has never before been described in a pelagic and highly

migratory seabird. Furthermore, the moult patterns of Cory's shearwater described in Chapter 6 were fundamental to interpret aspects of the adults' mercury dynamics addressed in Chapters 7 and 9.

To date, the use of blood has received little attention in monitoring or toxicological studies, despite its crucial role in proposed models for mercury dynamics in birds. Given the role of blood as internal carrier and ubiquous contact with other tissues, there is much information to be gained on avian mercury dynamics and monitoring value by investigating the kinetics of methylmercury in bird blood. This was undertaken in this study, with particular emphasis for Cory's shearwater, either by describing natural patterns in adults and chicks, in Chapter 7, or through experimental appraisals using free-living adults and chicks, in Chapters 9 and 10, respectively. The examination of natural patterns confirmed the major role of moult in governing mercury dynamics in adults and the need for knowledge of moult patterns to interpret concentrations in plumage. Natural patterns in chicks demonstrated a major growth dilution effect resulting from an imbalance between mercury dietary intake and co-accumulation in developing plumage and internal tissues. The experimental approaches produced an evaluation, for adults and chicks of varying age, of methylmercury half-times in blood, proportion of ingested methylmercury deposited in the blood volume, partition coefficients for blood:tissue concentrations and relationships between steady-state dietary intake of methylmercury and blood concentrations. These parameters showed lack of dose-dependency over a wide range of intakes and are an essential basis for a future quantitative compartment model. Elimination profiles of methylmercury in blood of adults comprise an initial fast phase that probably reflects the distribution of methylmercury in one compartment for which transport is characterised as flow limited (i.e. liver, kidneys and other target organs), and a second slow terminal phase that may reflect loss to a second compartment for which transport is diffusion limited (i.e. faeces, the intestinal lumen and plumage). Elimination profiles of methylmercury in blood of chicks were independent of age at exposure and showed that chicks excrete methylmercury much faster than adults as a consequence of rapid growth.

The dosing studies with free-living adult and young Cory's shearwater undertaken here also provided other types of information relevant for a more quantitative use of seabirds as monitors and for evaluation of methylmercury toxicity. Dose-response relationships between dietary methylmercury intake and levels in blood, eggs/hatchlings and plumage showed linearity over the wide range

of exposures employed. That provided a strong validation of the assumption that no saturation of the metal-accumulatory ability is operating in seabird tissues currently used as monitoring units. A general remarkable resemblance of dose-responses in blood of adults or small and large chicks, suggests a general dose-response kinetics for methylmercury in avian blood, governed basically by the volume of the body pool. Excretion rates of the 'pulse' of methylmercury confirmed the relative importance of plumage, eggs and faeces as pathways for mercury elimination, as found in previous studies. However, there was evidence of exogenous contamination of the plumage with mercury-containing exfoliated epidermal cells, that should be taken into account when interpreting excretion rates into the plumage by presumed endogenous incorporation of mercury. The relative contribution of current ingestion of mercury at the time of feather formation and mobilisation of mercury accumulated in internal stores during the inter-moult period to the final plumage burden is possibly the most uncertain aspect of mercury dynamics in adult birds. From the information gained here, it appears that in Cory's shearwater mercury excreted into the plumage more closely reflects current dietary intake. However, this may not be the case for most seabird species, that have more contracted moult periods. The doses administered to adult and young Cory's shearwater were assumed to be sub-toxic, and that was confirmed through monitoring of avian symptoms of methylmercury poisoning. Therefore, the exposure levels provide maximum avian non-observed-adverse-effect-levels (NOAELs) for external symptoms, which are extremely rare for wild birds.

Crucial for accurate monitoring of mercury using seabirds is a good knowledge of baseline 'noise' arising from methodological and biological factors that exert influence on tissue mercury concentrations. Such an examination was undertaken in Chapter 7 for potential monitor species in the Portuguese Atlantic. There, the natural variability in mercury concentrations among monitoring units and the confounding effects of factors as season, moult, sex or chick age were quantified to serve as basis for detection of genuine variation in environmental contamination. Results from that Chapter confirmed the importance of moult as an unwanted source of variation of levels in plumage and showed the existence of a general age-related decrease of mercury concentrations in chick plumage that needs to be taken into account in monitoring programs using chicks as monitors.

The wide natural variability of mercury burdens among monitor species prompted an investigation of their comparative dietary intake of mercury,

presented in Chapter 8. An *a priori* comparison of mercury concentrations in potential prey fish revealed a remarkable four-fold increase from epipelagic to mesopelagic fish, attributable to enhanced bioaccumulation in methylmercury enriched sub-thermocline waters. This finding provided a cue to understand the, to date, intriguing high burdens of mercury found in some seabirds, such as some petrels, which specialise on mesopelagic prey. Further, an operational link was confirmed in Sub-chapter 8.2 by a direct relationship between dietary mercury and seabirds' body burdens among fish/squid eating seabirds from the Azores. These findings add considerably to the potential of seabirds as monitors for mercury. The biomagnification of mercury concentrations between diet and seabird plumage represents the highest enrichment factor between consecutive trophic levels ever reported among biomonitors and emphasises further the uniqueness of bird feathers as monitoring units. In addition, ecological segregation in seabird communities have resulted in many parts of the world into feeding specialisation on epipelagic or mesopelagic organisms and offers a unique opportunity for easy and inexpensive monitoring of current geographical and historical variations in mercury contamination within and between those vertical compartments of the marine ecosystems.

Most of the Portuguese Atlantic represents a sub-tropical sector remote from continental anthropogenic emissions of mercury. Thus, it is suitable to investigate the global impact of human-related mercury inputs to the atmosphere at remote oceanic sites. That was undertaken in Chapter 11 using seabirds as monitors and integrating the knowledge acquired in previous chapters. Results on contemporary spatial patterns indicate a many-fold enhancement of mercury contamination in mesopelagic environments coupled with an even distribution of contamination in both compartments across most of the study region, from the remote mid-north Atlantic to coastal south-west Europe. The relatively uniform distribution of mercury contamination observed in the oceanic environment on a regional basis was interpreted as indicative of a major role of atmospheric deposition of elemental mercury associated with the global cycle. Microgeographic patterns found at the mainland coast correlate with patterns of increasing human activity and may be due to atmospheric deposition of particulate mercury emitted from local-scale sources. The historical trends of mercury contamination revealed increases over the last hundred years by two-fold near the apex of the epipelagic food web but comparative increases for the mesopelagic food webs rose four-fold. Whereas the long-term increase in the epipelagic environment is consistent with

current predictions and records of increase rates in the global background of mercury due to anthropogenic inputs to the atmosphere, the mesopelagic magnification of historical superficial increases is remarkable and brings scope for more research on the role of mixing into the ocean interior as a sink of anthropogenic-derived mercury.

The contemporary and historical patterns of mercury contamination in the Portuguese Atlantic provide an empirical linkage between increasing accumulation of methylmercury in aquatic organisms and anthropogenic influence in the global mercury cycle. Large increases, if substantiated further, especially in mesopelagic organisms, are of concern because of the current public-health problem resulting from widespread incidence of elevated levels of methylmercury in fish. In this respect, future monitoring is a priority and seabirds present good prospects for a comprehensive appraisal of the ecological hazards of global pollution by mercury into epipelagic and mesopelagic food webs.