Pathological Changes in Seals in Swedish Waters: The Relation to Environmental Pollution

Tendencies during a 25-year Period

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This thesis is dedicated to the memory of

my Mother and Father Hilda and Gustaf,

my Sister Gully, my Sister-in-law, Gunnel

and my Brothers-in-law,

Walter, Sabu and Nisse

ne buvez jamais d'eau

Abstract

This thesis concerns the disease situation for the three seal species that inhabit the Swedish coastal waters; the grey seal (*Halichoerus grypus*), the ringed seal (*Phoca hispida botnica*) and the harbour seal (*Phoca vitulina*).

A severe decline of the populations of Baltic grey and ringed seals took place during the second half of the 1960s. It was suggested to be caused by the contamination by industrial chemicals, above all organochlorines such as PCB and DDT. High concentrations of these substances were found in the Baltic biota.

The author has performed necropsy or examination of organ samples from animals, which were found dead on shore, by caught at fishery or killed by hunting during 1977-2002. Multiple chronic organ lesions were found most prominent in the female reproductive organs (uterine stenoses and occlusions), intestines (colonic ulcers) and adrenals (cortical hyperplasia). Severe lesions were present also in the skeleton, integument and kidneys. The character and distribution of the lesions was regular and the disease picture tentatively was named the Baltic Seal Disease Complex (BSDC). The changes in the female reproductive organs indicate that reproductive failure is an important factor behind the decline of the Baltic seal populations.

Adrenocortical hyperplasia was a regular and striking component of the BSDC. It is a common feature of prolonged stress in animals and man. The animals in this study have suffered from severe inflammatory processes in connection with more or less advanced malnutrition due to hampered ingestion and digestion of food. This is in the author's opinion the most probable explanation of the adrenal changes.

Inflammatory changes were most prominent in the intestines with deep ulcerations, in several cases leading to perforation of the intestinal wall. Bacteriological investigation revealed opportunistic or pathogenic micro-organisms but a common bacterial aetiology could not be suggested. The severity and wide dispersion of the lesions are interpreted as signs of a defective immune response. Minor lesions in the ileocaeco-colonic region caused by hookworms are regarded as the primary event of the ulcerous processes facilitating the establishment of secondary bacterial infections.

Harbour seals showed less developed pathological changes but instead were victims of two Distemper epizootics with high mortality (c60%), during 1988 and 2002. During the 14-year-period after 1988 the Swedish harbour seal population gradually attained to the pre-epizootic size; a fast recover compared with the situation in Baltic grey and ringed seal populations suffering from the BSDC problems.

A decrease in the prevalence of the lesions of the BSDC has been demonstrated concurrent with a decreased contamination of the Baltic biota towards the end of the 1900s. This is a strong indication of the role of pollutants as the main factor behind the BSDC. Other factors may also be involved, however, as indicated by the observation that the prevalence of intestinal ulcers still is high in Baltic grey seals.

Contents

Appendix

The present thesis is based on the following six papers (which will be referred to by their Roman numerals, see below) in which pathological changes in seals of the Swedish coastline during a 20 year period, 1977-1996 is described. In addition, and presented in different chapters under the headline ‘Observations during 1997-2002’, the pathological changes that occurred in these seal populations during the six subsequent years are included.

I. Bergman, A. & Olsson, M. 1985. Pathology of Baltic grey seal and ringed seal females with special reference to adrenocortical hyperplasia: Is environmental pollution the cause of a widely distributed disease syndrome? *Finnish Game Res. 44*, 47-62.

II. Bergman, A. 1999. Health condition of the Baltic grey seal (*Halichoerus grypus*) during two decades. Gynaecological health improvement but increased prevalence of colonic ulcers. *APMIS 107*, 270-282.

III. Bergman, A., Olsson, M. and Reiland, S. 1992. Skull-bone lesions in the Baltic grey seal (*Halichoerus grypus*). *Ambio 21*, 517-519.

IV. Lind, P.M., Bergman, A., Olsson, M. and Örberg, J. 2003. Bone mineral density in Baltic grey seal (*Halichoerus grypus*) in relation to pollution impact. *Ambio 32*, 385-388.

V. Bergman, A., Bergstrand, A. and Bignert, A. 2001. Renal lesions in Baltic grey seals (*Halichoerus grypus*) and ringed seals (*Phoca hispida botnica*). *Ambio 30*, 397-409.

VI. Bergman, A., Järplid, B. and Svensson, B.-M. 1990. Pathological findings indicative of distemper in European seals. *Veterinary Microbiology 23*, 331-341.

Abbreviations

ACH	adrenocortical hyperplasia
ADW	adrenal weight
AWC	aortic wall changes
BSDC	Baltic seal disease complex
CB	chlorobiphenyl
CB-126	chorobiphenyl congener[1]
CB-153	chorobiphenyl congener[1]
CD	Canine Distemper
CDV	Canine Distemper Virus
COLU	colonic ulcer
CWN	capillary wall nodules (kidney)
CWT	capillary wall thickening (kidney)
DDT	2,2-bis (4-chlorophenyl)-1,1,1-trichloroethane
FITC	fluorescein isothiocyanate
EM	electron microscopy
GALT	gut associated lymphatic tissue
GI	gastrointestinal
H&E	haematoxylin and eosin
LM	light microscopy
MSB	Martius scarlet blue
NVI	National Veterinary Institute
PALS	periarteriolar lymphocyte sheath
PAS	periodic acid Shiff
PASM	periodic acid silver methenamine
PCBs	polychlorinated biphenyls
PCR	Polymerase chain rection
PD	Phocine Distemper
PDV	Phocine Distemper virus
pQCT	peripheral quantitative computed tomography
SMNH	Swedish Museum of Natural History
SUAS	Swedish University of Agricultural Sciences
SWISP	Swedish investigation on seal pathology
TCP	tubular cell proliferations (kidney)
TCDD	tetrachlorinated dibenzodioxin
TCDD-TEF	tetrachlorinated dibenzodioxin-toxic equivalent factor

1Numbered according to Ballschmiter *et al*., 1993.

1. Introduction

The book Silent Spring by the American marine biologist Rachel Carson (Carson, 1962) is widely regarded as the main larm signal about environmental pollution and to have launched the present environmental movement. In Sweden, the negative impact of environmental contaminants on wildlife was first highlighted during the mid-1960s, linked with high concentrations of mercury compounds (Borg *et al.,* 1965; 1969; Berg *et al.,* 1966). The first species to signal about the serious pollution of the Baltic Sea was the white-tailed sea eagle (*Haliaeetus albicilla*) that by the mid-1960s reproduced at a strongly depressed rate; this has been linked with organochlorine contaminants (Helander *et al.,* 1982; 2002). It was later shown that a significant decrease in the number of offspring per successfully breeding eagle pair occurred already in the first half of the 1950s (Helander, 2003). As regards the seals, diving for food in Swedish waters, the problem with harmful environmental contamination began to be attended by scientists in the late 1960s (see below).

Historically, products from seals have provided an important extra income for people inhabiting the Swedish coast (Clark 1946, Almkvist *et al.*, 1980). According to a review by Hårding & Härkönen (1999) the Baltic was the main source of seal oil in Europe during the period 1300-1800. Seal oil was important in central Europe for the production of leather, soap, and paints. Seals successively lost their economic value at the end of the 19th century since cheaper alternatives to seal oil became available. At this time, by demand from fishermen, hunting of seals was encouraged in the Nordic countries by the introduction of effective bounty systems. Thus, seals have been subjects to intense hunting.

1.1. Situation in the Baltic

In the Baltic area bounties were paid in Sweden during the periods 1808-1864 and 1891-1967, in Finland 1909-1918 and 1924-1975 and in Denmark 1889-1927 and 1941-1977. At the end of the Second World War there were low numbers of seals in the area due to the co-ordinated effect of this hunting. The Swedish seal populations did not recover despite decreasing hunting pressure after the war (Bergman, 1956). Populations of all three species of seals, which inhabit the Baltic, the grey seal (*Halichoerus grypus*), the ringed seal (*Phoca hispida botnica*) and the harbour seal (*Phoca vitulina*), were found to further decrease rapidly in the 1960s and 1970s (Almkvist *et al.* 1980). The grey seal population in the Baltic decreased from 88 000 -100 000 at the beginning of the 20th century to approximately 4000 in the late 1970s (Hårding & Härkönen, 1999). The corresponding decrease for the ringed seal in the Baltic Sea during the same period was from 190 000 - 220 000 to approximately 5000 (Hårding & Härkönen, 1999). The harbour seal population in the Baltic Sea at the beginning of the 20th century amounted to about 5000 (Hårding and Härkönen, 1999).

Jensen *et al.* (1969) disclosed a serious DDT and PCB pollution of the Baltic. Hook & Johnels (1972) and Olsson *et al.* (1975) suspected pollution to be a factor involved in the decrease of the Baltic seal populations, and that this contamination was related to findings of aborted seal pups in the southern part of the Baltic. The main reason for the decrease of the Baltic seal populations after the Second World War and through the 1970s was reproductive failure. PCB compounds were suspected to be associated with interrupted pregnancies: high prevalence of uterine horn obstructions (stenoses and occlusions) was observed in Baltic ringed and grey seals in the 1970s (Helle *et al.*, 1976a, b). During that decade, the harbour seal population in the Dutch Wadden Sea was also reported to have decreased (Reijnders, 1976), and also in this decline PCBs were suspected to be the cause (Reijnders, 1980). In an experimental study, female harbour seals fed contaminated fish from the Dutch Wadden Sea showed lowered reproduction compared to seals fed fish that were less contaminated (Reijnders, 1986).

Slightly increasing populations of grey seals have been recorded in the Baltic from the mid 1980s. According to synchronised counts in 2007, performed in Estonia, Finland, Russia and Sweden, via aerial surveys and from boats and land, the countable Baltic grey seal population has amounted to about 22 000 individuals, a figure that include 1 049 in Bothnian Bay and North Kvarken, 1 834 in Bothnian Sea excluding the Åland archipelago, 8 515 in waters around south-western Finnish archipelago including the Åland archipelago, 6 349 in Swedish Baltic Proper between Gulf of Bothnia and 58°N (from northern limit of Fårö, Gotland to the landscape border Östergötland/Småland), 803 in the Gulf of Finland, 2 890 in waters west of Estonia, and 550 in Swedish Baltic Proper south of 58°N (Karlsson, 2007, personal communication).

Ringed seals occurred in most of the Baltic in the beginning of the 20th century, but are now confined to the Bothnian Bay, the Gulf of Finland, and the Gulf of Riga (Härkönen *et al.*, 1998). In recent years, counted numbers amounted to 4100 in the Bothnian Bay, 200-300 in the Gulf of Finland and 1400 in the Gulf of Riga (Härkönen *et al.*, 1998). Assuming that counted numbers comprise about 60% of the true population (Härkönen *et al.*, 1998), these figures suggest a total estimate at 10 000 ringed seals in the Baltic. According to results obtained during 2007 the size of the Baltic ringed seal population is approximately 8 000 animals (Härkönen, T., personal communication).

The Phocine distemper (PD) epizootic in 1988 had a limited impact upon the Baltic seal populations. Only harbour seals from the south-western part of the Baltic and a few grey seals, three aged females, were found to have died from this disease (Bergman *et al.* 1990). Likewise, during the PD epizootic in 2002 the Baltic Harbour seals suffered less than the Kattegat-Skagerrak populations (16% mortality) (Härkönen *et al.* 2004, in prep.). To the author's knowledge no Baltic grey seals were found to have died from PD during 2002. According to counts performed in 2003 the numbers of Baltic harbour seals were estimated at 1200 (Härkönen *et al.* 2006). Based on counts performed in 2007, the size of the Baltic harbour seal population amounted to 1 420 (Härkönen, T., 2007, personal communication).

1.2. Situation at the Swedish west coast

As mentioned above legal hunting and bounty systems affected the Kattegat-Skagerrak seal populations almost to the end of the 1970s. The grey seal population in the area amounted to c. 1000-2000 during the 1890s and was virtually exterminated during the 1930s and 1940s and comprised c. 25 animals in the 1980s (Heide-Jørgensen and Härkönen, 1988). During the epizootic in 1988 four grey seals died with signs of this disease (Heide-Jørgensen and Härkönen, 1988). There is no recent change in the size of the grey seal population in the area (Härkönen *et al.*, 2007a).

Based on official bounty payment statistics Heide-Jørgensen & Härkönen (1988) calculated the size of the Kattegat-Skagerrak population of harbour seals during 1890-1987. From a number of c. 17 000 individuals in the 1890s, the population size rapidly decreased to c. 2000 in the 1920s, a number which remained rather constant up to 1980. This decrease was a result of intense hunting, especially in the early part of this period. Bounties were paid in Denmark during 1899-1927 and 1946-1970, and in Sweden during 1902-1965. During the 1980s the population increased in size, to more than 8000 individuals (Heide-Jørgensen & Härkönen, 1988). This positive tendency however suddenly changed because of the 1988 Phocine distemper (PD) epizootic in north-western Europe. This disease, resulting in about 60% mortality, reduced the harbour seal population to about 3000 individuals (Heide-Jørgensen *et al.* 1992). During the following years the population increased at more than 12% per year and amounted to 23 000 in the spring of 2002, when a new outbreak of PD occurred (Jensen *et al.* 2002). This time mortality rates showed considerably larger variations at the Swedish west coast and the Baltic. Only 24% died in the Danish parts of the Kattegat, whereas 44% died in Swedish parts of the area (Härkönen *et al.* 2006.). Highest mortality rates (66%) were observed in the Skagerrak, while, as said above, the Baltic harbour seals only suffered 16% mortality (Härkönen *et al.* 2006). During the summer of 2007 a new contagious disease appeared in the West coast population of harbour seals. It was judged as a respiratory tract infection of, at the time of writing, unclear aetiology (Härkönen *et al.* 2007b). Population surveys performed in August 2007, when reported victims of this year epizootic were few, showed an estimated size of the population to 15 300 (Härkönen, et al., 2007b). Since projected numbers (at 12% annual increase) were 17 600, it is suggested that total mortality up to the end of August was 2 300 seals. This recent estimated size of the population reflects especially the epizootic during summer and autumn 2007 and to a lesser extent the epizootic during 2002 (Härkönen, *et al.,* 2007b).

1.3. The start and aim of the present study

A co-operation between specialists at the Swedish Museum of Natural History in Stockholm and the Department of Veterinary Pathology, Swedish University of Agricultural Sciences, Uppsala, was initiated in 1977. Since then seals found dead on the shoreline or found drowned in fishing gear along the Swedish coasts have been the subjects of post-mortem studies. In the following this investigation project is referred to as the Swedish investigation on seal pathology (SWISP). Post-mortem whole body or organ sample examinations was made by the author on most grey seals, ringed seals and harbour seals from Swedish waters sent to the Swedish Museum of Natural History over the period 1977-2002.

As found in earlier studies (Helle *et al.*, 1976a, b), results also from the SWISP project revealed a high prevalence of uterine occlusions and stenoses in Baltic grey and ringed seals (Bergman & Olsson 1985). Furthermore, uterine tumours of smooth muscle cell origin (leiomyomas) were found to be common in Baltic grey seals (Bergman & Olsson 1985). Certain chronic lesions in non-reproductive organs were also found to be common in both sexes of these species (Bergman & Olsson 1985 and 1989). Taken together the lesions present in the seals have been referred to as the Baltic seal disease complex (BSDC) (Bergman, 1999, Bergman *et al.* 2001).

A morphologic description of the main specific organ lesions occurring in the Baltic seals was the first aim of this investigation (Bergman & Olsson 1985 and 1989). A second aim was to try to elucidate the etiology and pathogenesis behind the BSDC. A probable connection between the BSDC and exposure to environmental contaminants is a continuous subject for the discussions in the papers presented here. The thesis is focused on grey seals since a relatively large number of individuals of this species have been investigated compared to that of ringed seals and harbour seals. The size of the Baltic grey seal material made a statistical investigation possible as regards the prevalence of organ lesions of the disease complex and the temporal variation of concentrations of contaminants in Baltic biota (Bergman, 1999).

The disease problem in Baltic seals during the latter half of the 20th century has a character of an insidious onset and course. The silent chronic character of disease in seals in Swedish waters abruptly changed in 1988 due to the epizootic that affected harbour seals and grey seals in waters of north-western Europe, causing mass mortality in harbour seals (Dietz *et al.* 1989). A third and important aim of the SWISP project was to try to reveal the cause of this epizootic.

1.4. The three seal species, a presentation

1.4.1. Biological data

As a background for further reading some biological data and characteristics on the three species of seals that inhabit the Swedish waters are shown. The data in Table 1 and Table 2 and in the running text below are compiled via records from investigations at the Swedish Museum of Natural History, e.g. Almkvist *et al.* (1980) and from Hewer (1974), Bonner (1979), King (1983) and Härkönen & Heide-Jørgensen (1990). Blubber thickness is determined in the median ventral thoracic region. Body length is the so-called total length (nose-tip of tail).

The field photographs of the three species (Figs. 1-3) show fur appearance just after moult in grey seals (Fig. 1) and during moult in harbour seals (Fig. 3). As a rule, the fur of females of grey seals looks lighter than that of males (Fig. 1).

Table 1. *Some biological data on seal species in Swedish waters.*

	Grey seal *Halichoerus grypus*		Ringed seal *Phoca hispida botnica*		Harbour seal *Phoca vitulina*	
	Female	Male	Female	Male	Female	Male
Length of life, years	≤42	≤25	≤ 35	≤35	≤ 34	≤ 32
Age at sexual maturity, years	3-5	4-8	4-6	5-8	3-4	5
Body weight, kg	≤200	≤ 300	≤ 110	≤ 125	≤ 95	≤ 105
Blubber thickness, cm	2.5-6		2.5-8		2-4.5	
Body length, cm	≤ 200	≤ 300	≤ 150	≤ 170	≤ 170	≤ 180
Moulting period	May-June		May-June		mid July–mid September	

Fig. 1. Grey seals at Kalhällan, Agö, Landscape of Hälsingland, Bothian Sea, July 11, 1995 (just after moult). Light-coloured female (left), male (middle) and female (right). Photo: Erik Isakson, Contaminant research group, SMNH.

Fig. 2. Ringed seals at Southwestern archipelago of Finland. Photo: Seppo Keränen, Pori, Finland.

Fig. 3. Harbour seals at Stora Drammen, Koster, Landscape of Bohuslän, Skagerrack, August 31, 1998 (during moult). The animals are changing colour of fur from brown to grey. Photo: Erik Isakson, Contaminant research group, SMNH.

1.4.2. Period of birth, lactation and oestrus/mating

In grey seals this period lasts for 2-3 weeks and occurs during the last 3 weeks of February - 1st week of April. Most births occur in the 1st 2 weeks of March and oestrus/mating during the last week of lactation.

In ringed seals the period lasts for 4-8 weeks and occurs during the 2nd week of February – March. Most births occur in the beginning of March and oestrus/mating near end of lactation. In ringed seals the lactation period is affected by ice conditions, such that it is shorter during mild winters compared with severe winters, when the break-up of ice fields occurs later (Härkönen, personal comm.).

In harbour seals the period lasts for 6 weeks and occurs during 4th week of May – 1st week of July, peaking at 20 June. The lactation lasts for 23 days and mating during the last week of lactation.

1.4.3. Season of female reproduction

The time between fertilisation and blastocyst implantation is c.100 days in grey seals, 80-100 days in ringed seals and c.100 days in harbour seals. This period is referred to as the period of delayed or suspended implantation of the blastocyst in the uterus. The following period of so-called active gestation (embryonic and foetal growth to parturition) lasts for c.240 days in grey seals, c.270 days in ringed seals and c.240 days in harbour seals. In total, the duration of gestation is c. 340 days in all the three species.

Blastocyst implantation, after which pregnancy can be confirmed, occurs during mid June-mid August in both grey seals and ringed seals, and from mid October in harbour seals.

Fig. 4. Grey seal female and pup at Forsmark Breeding Station for grey seals, Sweden. Photo: Anna Roos, Contaminant research group, SMNH.

1.4.4. Offspring and lactation

Grey seal pups: Weight gain during lactation: 1.2-1.5kg/day. Mother's milk contains c. 52% fat. Embryonic fur persists at birth and begins to shed after 3 weeks. The pup moult is complete in another 4 - 5 days.

Ringed seal pups: Weight gain during lactation: 0.3 kg /day. Mother's milk contains 45-55% fat. Embryonic fur persists at birth and begins to shed after 2 weeks and is complete within 6 weeks.

Harbour seal pups: Weight gain during lactation: 0.5 kg /day. Mother's milk contains c. 45% fat. Embryonic fur is shed in uterus, or latest, at birth.

Note the high fat content of mother's milk, implying that considerable amounts of toxic lipophilic compounds from the mother's fat depots may be transferred to the pup during the short period of lactation.

Table 2. *Some data on offspring (pups) of seals in Swedish waters.*

	Grey seal	Ringed seal	Harbour seal
Body weight, newborn	8-10 kg	4-5 kg	c 8.7 kg
Body length, newborn	60-100 cm	60-70 cm	c 80 cm
Weight at end of lactation	35-60 kg	15-18 kg	c 22 kg

1.5. Composition of diets

There are two reports on the diets of Baltic seals, Söderberg (1975) and Lundström *et al.* (2007). Söderberg's report is comprehensive and comprises all the three Baltic species. The report by Lundström *et al.* deals with Baltic grey seals only. In both reports cited food composition was investigated by fish prey species identification of otoliths present in gastrointestinal (GI) tracts. Otoliths (ear-stones) from fishes are species-specific, hard to break down and useful for fish diet examination in seals. Fish species occurrence in the seals was expressed as follows. For each species of fish, the proportion (%) between the numbers of GI tracts containing its otoliths and the total number of the gastrointestinal tracts investigated was calculated. In the following, the percentages so obtained are called GI tract proportions. To avoid confusion as regards the percentages presented below it must perhaps be said that each GI tract, as a rule, contained otoliths from more than one fish species. The two investigations give no information about weights of the fishes consumed.

Härkönen (1987) and Härkönen & Heide-Jørgensen (1991) studied the diet of harbour seals in the Kattegat-Skagerrak area by investigating fish otoliths obtained from faeces collected at seal haul-outs. In these investigations prey fish weights were calculated via sizes of otoliths.

Scientific names of the fish prey species are according to Kullander (2003).

1.5.1. Baltic grey seals

Söderberg (1975) investigated a material collected from March 1968 to June 1971 and listed remnants (otoliths) of 20 fish species. Similarly, Lundström *et al.* (2007) investigated a material of GI tracts from grey seals collected during 2001-2004 and found otoliths or other hard structure parts from 24 prey fish species possible to identify. Most of the data on frequency of occurrence in GI tracts of grey seals recorded by Söderberg (1975) and Lundström *et al.* (2007) are shown in Table 3.

Table 3. *Frequency of occurrence (%) of remnants of different prey fish spp. in gastro-intestinal tracts of Baltic grey seals collected during 1968-1971 (Söderberg, 1975) and 2001-2004 (Lundström et al., 2007).*

	Fish species	1968-1971	2001-2004
Clupeoids	Herring (*Clupea harengus*)	23.5%	80.7%
	Sprat (*Sprattus sprattus*)	3.5%	26.9%
Gadids	Cod (*Gadus morhua*)	21.0%	4.1%
Salmonids	Salmon (*Salmo salar*)	12.5%	4.1%
	Sea trout (*Salmo trutta*)	7.0%	3.5%
	Salmon or trout		4.1%
Salmoniforms	Whitefish (*Coregonus oxyrinchus*)	4.2%	20.0%
	Vendace (*Coregunus albula*)	0.7%	
	Smelt (*Osmerus eperlanus*)	3.5%	4.1%
Flat fish	Dab (*Pleuronectes limanda*), Turbot (*Psetta maxima*), Flounder (*Platichthys flesus*), Plaice (*Pleuronectes platessa*)	12.6%	5.5%
Sculpins	European sculpin (*Myoxocephalus scorpius*), Lucky proach (*Taurulus bubalis*), Fourhorn sculpin (*Triglopsis quadricornis*)	5.6%	2.8%
Anguillids	Eel (*Anguilla anguilla*)	5,6%	1.4%
Esocids	Pike (*Esox lucius*)	3.5%	1.4%
Percids	Perch (*Perca fluviatilis*)	4.9%	4.1%
	Ruffe (*Gymnocephalus cernuus*)	2.1%	2.1%
	Pikeperch (*Sander lucioperca*)		0.7%
Cyprinids	Roach (*Rutilus rutilus*)		10.3%
Zoarcids	Eelpout (*Zoarces viviparous*)	2.1%	6,9%
Gobids	Two-spotted goby (*Gobiusculus flavescens*)	1.4%	1.4%
Ammodytids	Sandeels (*Ammodytes* spp.)	1.4%	6.9%
Lotids	Burbot (*Lota lota*)		2.8%
	Lumpsucker (*Cyclopterus lumpus*)		0.7%
	Four-bearded rocking (*Encheliopus cimbrius*)		0.7%

An evident change of the grey seal diet has occurred during the last decades, when comparing the situation in 1968-1971 (Söderberg, 1975) and 2001-2004 (Lundström *et al.* 2007). The contribution of herring species to Baltic grey seals has increased, and cod, an earlier common prey species, is nowadays rare in their diet (Table 3).

1.5.2. Baltic ringed seals

Twelve fish species and the sandeel family (Ammotids) were listed in the investigation of Baltic ringed seals 1968-1971 (Söderberg, 1975). GI tract proportions were as follows. Herring fish species: herring (19%). Cod (7.7%). Salmonids: salmon (9.6%), sea trout (3.8%), whitefish (11.5%), vendace (3.8%) and smelt (1.9%). Sculpins: fourhorn sculpin (13.5%) and European sculpin

(9.6%). Other fish species: eel (3.8%), ruff (5.8%), blenny (5.8%) and sandeels (5.8%). The GI tracts of the ringed seals also contained Molluscs: common mussel (5.8%), and Crustaceans: opossum shrimp, *Mysis relicta* (17.5%) and sea slater sp. *Saduria entomon* (21%). Crustaceans were found in more than 25% of the GI tracts in ringed seals while in none of the grey seal's. Their occurrence in ringed seals was strictly limited in time viz. during March, April and May. Crustaceans must be regarded as a form of "emergency" food during the season of birth, lactation and moult (Söderberg, 1975). Also arctic ringed seals feed on crustaceans during the corresponding period (Mansfield, 1963). It should be mentioned that both the two crustaceans and the fourhorn sculpin, which all are important food for the ringed seal, are like the ringed seal itself considered to be glacial relicts in the Baltic (Söderberg, 1975).

1.5.3. Baltic harbour seals

A small material of Baltic harbour seals was collected during 1968-1971 (Söderberg, 1975). Five fish species were listed. GI tract proportions of these species were as follows. Cod (14.5%), Salmonids: whitefish (14.5%). Flat fish species: flounder (29%) and turbot (14.5%). Other fish species: eel (43%). The GI tracts of the harbour seals also contained Molluscs: common mussel (14.4%). There is no report on the present food composition in ringed seals and harbour seals of the Baltic.

1.5.4. Harbour seals in Skagerrak-Kattegat area

Härkönen (1987) studied prey fish otoliths from faeces collected at harbour seal haulouts at a rocky shore habitat at the Koster islands in Skagerrak and at a sandy shore habitat, the island of Anholt in Kattegat, during 1977-1980. He also calculated fish weights via otolith sizes. Such an investigation was also carried out in 1989 on harbour seals in Skagerrak (Härkönen & Heide-Jørgensen, 1991).

In the 1977-1980-investigation harbour seals in Skagerrak were found to prey on 20 fish species (Härkönen, 1987). Three families of fish: cod fish species, flatfish species, and herring species were predominant. Cod species represented 50%, flat fish species 26% and herring species 8% by fish weight. The following species were the main food by weight at this habitat. Herring fish species: herring (7%) and sprat (1%). Cod fish species: cod (20%), poor cod, *Trisopterus minutus* (7%), whiting, *Merlangius merlangus* (6%) and Norway pout, *Trisopterus esmarkii* (6%). Flat fish species: lemon sole, *Microstomus kitt* (15%) and long rough dab, *Hippoglossoides platessoides* (7%). Other fish species: sandeels, Ammodytids (7%).

In Kattegat harbour seals preyed on 12 species. The flat fish species (pleuronectides): dab, plaice, *Pleuronectes platessa*, and flounder made up 75% of the diet and sandeels 9.3%, while cod species (15.5%) were less important in this habitat (Härkönen, 1987).

Harbour seals in the Skagerrak investigated in 1989 preyed on 31 species of fish but six species: herring, cod, blue whiting (*Micromesistius poutassou*), whiting, Norway pout and lemon sole constituted the bulk of the diet (Härkönen & Heide-Jørgensen, 1991). This diet was almost the same as found in the 1977-80-study, with the exception that the proportion of herring was more than doubled in 1989 (Härkönen & Heide-Jørgensen, 1991).

1.5.5. Daily food consumption

It is difficult to determine the daily food consumption in wild-living seal populations. Available data are from experimental studies using captive animals maintained at activity levels approximating those occurring in their natural environment (Mohn & Bowen, 1996).

Grey seals

Data can be summarized as follows. Mohn & Bowen (1996) modelling the impact by grey seals in Canada on Atlantic cod calculated an ingestion of fish prey around 5kg/day for animals weighing 50-100kg, 5-6 kg/day when weighing around 150 kg, and up to 8-9 kg/day when weighing around 250 kg/day.

Harbour seals

Based on data from harbour seals obtained during 1977-79 (Härkönen, 1987) and in 1989 (Härkönen & Heide-Jørgensen, 1991) on measurements of seasonal changes in mass and on estimates of the energy required for maintenance and growth, average daily energy requirement was calculated as 4680 kcal per seal that corresponds to a daily consumption of 3.67-4.15 kg fish per seal depending on diet composition (Härkönen & Heide-Jørgensen, 1991). Total consumption of fish was proportional to population size and was in the magnitude of 2400 tons in 1989 and the consumption of commercially important fish species did not exceed 1% of the catches of these species by Skagerrak fishery (Härkönen & Heide-Jørgensen, 1991).

Ringed seals

The data in Chapter 1.5.2 indicate that the ringed seals consume a lot more of crustaceans than the grey and the harbour seals. However, no detailed data on the daily food consumption in wild living populations have been found.

2. MATERIAL AND METHODS

2.1. Overview of the animals described in Paper I-VI and under 'Observations during 1997-2002'

The material from the Baltic and Swedish west coast area described in this thesis: Paper I-VI and 'Observations during 1997-2002' is compiled in Table 4. and included whole bodies and organ samples of a total of 729 Baltic greys seals (376 whole bodies and 353 organ samples respectively), 69 Baltic ringed seals (55 and 14 respectively) and, 65 harbour seals (61 and 4 respectively) from the Swedish west coast (Table 4).

Table 4. *Number of grey seals, ringed seals and harbour seals from different populations presented in Papers I-VI and in 'Observations 1997-2002', grouped by age: Yearlings: <1-year-olds, sub-adults: 1-3-year-olds, and adults: ≥4-year-olds. Number of animals subjects to whole body examination (W) and organ sample examination alone (S).*

			Grey seals				Ringed seals			Harbour seals			
			Baltic		Atlantic		Baltic		Arctic	Swedish w. coast		Baltic	
			W	S	W	S	W	S	W	W	S	W	S
I	BSDC[1]	<1	2	0	0	0	2	0	0	0	0	0	0
	♀♀	1-3		0	0	0	0	0	0	0	0	0	0
		≥4	13	42	0	0	8	14	0	0	0	0	0
II	BSDC	1-3	63	1	0	0	0	0	0	0	0	0	0
	tendency ♀♀♂♂	≥4	82	13	0	0	0	0	0	0	0	0	0
III	Skull bone Lesions ♀♀♂♂	≥6	0	234	0	165	0	0	0	0	0	0	0
IV	Bone density ♂♂	≥4	0	43	0	0	0	0	0	0	0	0	0
V	Renal lesions	<1	10	0	3	0	2	0	0	0	0	0	0
	♀♀♂♂	1-3	27[3]	0	8	0	1	0	2	0	0	0	0
		≥4	44[4]	0	0	0	26	0	21	0	0	0	0
VI	PD[2] 1988	<1	0	0	0	0	0	0	0	13	0	0	0
	♀♀♂♂	1-3	3	0	0	0	0	0	0	12	0	0	0
		≥4	9	0	0	0	0	0	0	12	0	0	0
	Observations	<1	43	6	0	0	6	0	0	2	0	5	0
	1997-2002	1-3	20	5	0	0	8	0	0	12	0	3	0
	♀♀♂♂	≥4	52	9	1[5]	0	2	0	0	10	4	2	1
	S^n		368	353	12	165	55	14	23	61	4	10	2

1) BCDC: Baltic seal disease complex; 2) PD: Phocine distemper, 3) Includes 1 male from a Swedish zoological garden, 4) Includes 4 males from a Swedish zoological garden, 5) a male from the Swedish west coast.

One grey seal, belonging to the Atlantic population, was collected on the Swedish west coast in 2002 (Table 4). As reference material served skull bones from 165 and whole bodies from 11 Atlantic grey seals as well as whole bodies from 23 arctic ringed seals (Table 4). The study thus involves 864 seals and in addition 199 seals were used as reference material; in total 1063 seals. For more information about the material examined in Paper I-VI and during 1997-2002, see Ch. 2.11-2.17.

2.2. Animals from Swedish and Finnish waters, ways of collection, necropsies

A large number of grey seals collected in the Baltic and the Bothnian Bay, a few from Öresund and one from the Swedish west coast have been examined during the course of the project. In addition some grey seals from Swedish zoological gardens were investigated. Ringed seals were collected in the Bothnian Bay and the Baltic, and harbour seals in the southern part of the Baltic, Öresund and on the Swedish West Coast. The comparatively large number of grey seals that have been investigated has made trend over time studies and statistical tests possible in this species. All six papers deal with grey seals, two with ringed seals (Paper I and V) and one with harbour seals (Paper VI). Records in 'Observations during 1997-2002' concern all the three species.

The animals were found dead on shores or found drowned in fishing gears. A few grey seals were shot due to severe illness. During 1997-2002 the material also concern Baltic grey seals and harbour seals on the Swedish West Coast obtained at a limited legal hunting authorised by the Swedish Environmental Protection Agency and a few grey seals killed illegally.

Necropsy and organ sample examination

Complete necropsy was performed by the author on animal carcasses in conditions fresh enough to allow adequate judgment of macroscopic changes. A summary of the total number of animals subjects of whole-body investigations of the three actual species necropsied during the whole investigation period 1977-2002 is shown in Tables 5 and 6. When reading these tables it should be remembered that few <1-year-olds were included in Paper I-VI.

Sampling of female reproductive organs (Paper I) and other organs (Paper II) from animals found dead on shore were collected by two field collaborators in Sweden who were thoroughly instructed in necropsy hygiene and technique. Besides bones obtained at necropsy the collection of bones at the Vertebrate Section, SMNH was used (Paper III and IV).

Necropsy procedures included determinations of body and organ weights, body length, other body measurements and blubber thickness (nutritional state). Tissue samples were saved for histological examination, analysis of environmental contaminant concentrations and, when appropriate, for microbiological and parasitological examination.

A problem at necropsies of animals belonging to wild-living populations is often a high-degree of autolysis. The animals dealt with in this project are with few exceptions obtained at fishery or found dead at shore. For the first-mentioned animals the time between finding and necropsy can be determined but for the last-mentioned not. At necropsy a rather subjective way must be used to determine whether the material may be suitable for histology or not. Generally, when the degree of autolysis was judged to allow valuable histological information, sampling for bacteriological examination also was considered. The shortest time between finding and necropsy, as a rule was around 48 hours. An exception regards part of the female Baltic ringed seals. Eleven of them were collected at Simo in the Northern Bothnian archipelago in November 1978. Both air- and water temperature were low at this field expedition and the time between death of the animals and necropsy did not exceed 24 hours.

Table 5. *Number of whole body examinations on yearlings of seals from Swedish waters during three consecutive periods.*

Period	Grey seal		Ringed seal		Harbour seal	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
1977-1986	6	11	4	1	2	0
1987-1996	54	58	4	4	13	14
1997-2002	26	19	1	5	2	3
S^n♀♀/♂♂	86	88	9	10	17	17
S^n♀♀+♂♂	174		19		34	

Table 6. *Number of whole body examinations on ≥1-year-old seals from Swedish waters during three consecutive periods.*

Period	Grey seal		Ringed seal		Harbour seal	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
1977-1986	24	22	12	8	9	6
1987-1996	44	61	10	4	18	30
1997-2002	12	61	7	3	12	20
S^n♀♀/♂♂	80	144	29	15	39	56
S^n♀♀+♂♂	224		44		95	

2.3. Reference animals

Reference materials were obtained from marine environments that are less polluted than the Baltic area. Bone samples from the Baltic collected before the increase of concentrations of pollutants were also used.

Grey seals for comparisons (Paper V) were necropsied during a hunting expedition to Helmsdale in northern Scotland during April 19-26, 1988. Ringed seals for comparison (Paper V) were necropsied during a hunting expedition to King’s Bay, Svalbard during June 3-20, 1981.

Skull bones from Baltic grey seals collected 1850-1955, before the main introductions of organochlorines in the environment were studied in the investigations described in Papers III and IV). A large skull bone material of Atlantic grey seals from the North Sea area and the North Atlantic area around the British Isles was also included (Paper III) as well as a few skulls from the Swedish West Coast (Paper IV).

Further information on the different materials is given below under respective Paper. For an overview of the number of animals subject to necropsy and organ sample investigations, see Table 4.

2.4. Age and Age classes

Age determination

Age determination was performed according to Johnston & Watt (1981) by examination of the annual growth pattern in cementum zones in undecalcified tooth sections, about 50 mμ thick, viewed under bright-field, polarised light and with a compensator G Rot I (Zeiss).

In two necropsy cases, one grey seal and one ringed seal (Paper II), teeth were not available for age determination, why age was estimated. This was done by studying body size and weight, the development of reproductive organs and the degree of thymic involution. Tissue of the thymus has not been observed grossly in Baltic seals older than 20 years (author's observation).

Rough age classification of grey seal skull bones (Paper III) was done by examination of size and morphology of skulls and teeth.

Age classes

The animals were grouped by age into classes as shown in Table 4. The basic age classes are yearlings (<1-year-olds), subadults (1-3-year-olds) and adults (≥4-year-olds). The youngest grey seal females from the Baltic found to be pregnant were 4 years old. This age limit agrees well with the compiled information on sexual maturity presented in the Introduction, Table 1, and was set as the lowest limit for adults in five of the six reports (Table 4).

In the study of skull bones from grey seals (Paper III), a lowest age limit for adults was set at six years as a security measure since age was only roughly estimated (Table 4).

Different age groups and age limits are presented in each paper. See also Table 4.

2.5. Semiquantitative evaluation

Both at macroscopic and light microscopic investigations semiquantitative evaluations were used as regards the degree and extent of lesions. In Paper II and V a four-degree scale was used: Zero, no or non-evident changes; one, slight; two, moderate; and three, severe. The grades zero and one were pooled in Paper II, concerning a trend over time investigation of lesions of the BSDC, so also the grades two and three. In paper III, a three-degree scale was used: Zero, no changes; one, slight; and two, severe.

2.6. Light microscopy (Paper I, II and V)

Small but representative pieces of organs were fixed in 10 % neutral buffered formalin and embedded in paraffin. Sections, about five microns thick, were stained routinely with haematoxylin and eosin (H & E) and. When appropriate other staining techniques were used, including van Gieson, periodic acid Shiff (PAS) technique, periodic acid silver methenamine (PASM), Masson´s trichrome, Ladewig´s modification of Mallory´s trichrome staining, Martius-Scarlet-Blue (MSB) method for fibrin, Verhoeff`'s and Weigert's methods for elastic fibres, van Kossa's method for calcium and Congo Red for amyloid. As special staining for viral inclusions the Shorr technique (1941) modified by Page and Green (1942) was used. Frozen sections were stained for lipids with Sudan IV. For further information on the staining techniques, see Bancroft & Stevens (1982).

2.7. Electron microscopy (Paper V)

One-mm. thick slices of renal tissue were fixed in 1.5 per cent glutaraldehyde in 0.1 M cacodylate buffer for 48 hours. Post-fixation was performed in 1 per cent osmium tetroxide in the same buffer for two hours at 4oC followed by dehydration in alcohol-acetone and embedding in Lx 112. Ultra-thin sections were stained with uranyl acetate and lead citrate.

2.8. Bacteriology

Bacteriological examination was considered as a part of the post mortem examination when bacteriological infection was suspected. Since results in bacteriology concerning this material are not reported earlier the frequency of the sampling is based on the total number of necropsies performed 1997-2002 (Tables 5 and 6).

A total of 106 animals (18 yearlings and 88 ≥one-year-olds: Table 7) of the three actual species were subjected to bacteriological examination that is 18 % of the total of 590 seals (227 yearlings, Table 5) and 363 ≥one-year-olds (Table 6) subjected to whole body examinations.

For different organs an overview of the number of animals and the distribution of the total of 264 samples examined can be read from Table 8. The samples from female reproductive organs primarily comprise the uterus and uterine wall. Within this category samples from macroscopically normal uteri of two grey seals and three ringed seals are included. The samples from the gastro-intestinal tract primarily are from intestinal ulcers, intestinal wall and regional lymph nodes. The samples described below under "other organs" also include abscesses and abscess walls.

Table 7. *Number of yearlings (pups and post pup-stage) and older animals of the three seal species in Swedish waters subjects of bacteriological examination during 1977-2002.*

Species	Sex	^{n}Pups	^{n}Post pup-stage	$^{n}\geq$One-year-olds	^{Sn}♀♀+♂♂
Grey seals	♀♀	4	4	24	
	♂♂	6	1	28	67
Ringed seals	♀♀	0	1	13	
	♂♂	0	0	3	17
Harbour seals	♀♀	0	1	8	
	♂♂	0	1	12	22
S^{n}		18		88	106

Table 8. *Number of animals and samples from different organs of the three seal species in Swedish waters, subjects of bacteriological examination 1977-2002.*

		Grey seals		Ringed seals		Harbour seals	
		^{n}animals	^{n}samples	^{n}animals	^{n}samples	^{n}animals	^{n}samples
Reproductive	♀♀	17	24	12	25	2	4
organs	♂♂	1	1	0	0	0	0
Intestine	♀♀	10	26	3	6	0	0
(colonic ulcers)	♂♂	10	17	0	0	0	0
Other organs	♀♀	28	41	4	5	7	14
	♂♂	27	67	3	7	12	27

The samplings were done using sterile techniques by means of sterile instruments for each new level of the surfaces of the carcass to avoid contamination during the autopsy as well as for sampling the tissues to be examined. The samples were transported in sterile Petri dishes for examination to the Dept. of Clinical Microbiology, Swedish University of Agricultural Sciences (SUAS) or the Dept. of Bacteriology at the National Veterinary Institute (NVI) in Uppsala, Sweden.

Until the beginning of 2001 almost all bacteriological sampling was performed at the Dept. of Pathology, SUAS. Some of the sampling was performed at the SMNH, transferred to transport media for aerobic and anaerobic bacteria (Culturette; Copan innovation, Italy) and sent to actual laboratory.

Generally the samples included were the primary focus of infection and its regional lymph node and also other tissues if bacteraemia was suspected. The bacteria isolated were identified according to standard procedures at the SVA and basically as described by Quinn *et al.* (1994).

2.9. Parasitology

The animal material included 38 grey seals, nine ringed seals and 17 harbour seals, altogether 64 animals. The grey seals were 16 females and 22 males, the ringed seals six females and 3 males, and the harbour seals nine females and eight males. The material of both sexes of the three species included post-pup stage yearlings as the lowest age. The highest age of grey seal females and males was 41 and 23 years respectively. The oldest ringed seal female was 12 years. The ringed seal males included two yearlings and one with unknown age. Of the harbour seals the oldest female was 35 years and the oldest male 22 years.

Signs of parasite infection were found predominantly in the stomach, intestine, liver and lung and exceptionally in other tissues. Besides, the diaphragm was sampled for detection of trichina.

Material for parasitology was sent to the Dept. of Parasitology, National Veterinary Institute (NVI), Uppsala, and was examined in accordance with general methods for parasitological examinations (Anon. 1986). Organs or parts of organs sent for parasitology were inspected macro- and microscopically. Scrapings were made from mucous membranes and inspected under a microscope. Lungs, liver, stomach and intestine were cut open for inspection. The contents of the stomach and small intestine were washed through a sieve with a mesh size of 100 µm and the remains were inspected under a microscope. The muscular part of the diaphragm was digested for examination of larvae of *Trichinella*. Isolated parasites were identified to species if possible (Anon. 1986).

2.10. Virology

During the course of the Swedish seal project in pathology focus in virology exclusively has concerned the two outbreaks of Phocine Distemper (PD) in 1988 and 2002. During the 1988 PD epizootic diagnostic tools in virology to reveal Phocine distemper virus (PDV) was developed (Osterhaus and Wedder, 1988).

After 1988 PD diagnostic methods have improved and also comprise: *Immunofluorescence assay*: PDV-antigen in acetone-fixed cryostat-sections of different tissue specimens was detected by an indirect immunofluorescence assay. Shortly, a monoclonal antibody, (designated 1.280E7, kindly supplied by Dr. Claes Örvell, Stockholm) was used in a dilution of 1/100. The antibody is directed against the nucleocapsid protein and reacts with a range of canine distemper and phocine distemper viruses (Blixenkrone-Møller *et al.*, 1992). A fluorescein isothiocyanate (FITC) conjugated sheep-anti-mouse IgG serum was used to detect the bound monoclonal antibody.

Polymerase chain rection (PCR) assay. A universal morbillivirus PCR was used on seal tissue samples directed against a highly conserved region of the morbillivirus phosphoprotein gene as described previously (Barrett *et al.*, 1993).

2.11. Paper I. Description of disease complex in Baltic grey- and ringed seals

Females of grey and ringed seals were described in this report. With one exception, an adult grey seal from a Swedish zoological garden were they collected in the Baltic proper and the Gulf of Bothnia during 1977-1983.

Necropsy was made on 19 grey seals: two yearlings, four subadults and 13 adults (Paper I: Table 2) and sample examination of reproductive organs from further 42 adults (Paper I: Table 1). Of a total of 24 ringed seals necropsy was made on ten animals: two yearlings and eight adults (Paper I: Table 2) and sample examination of reproductive organs from further 14 adults (Paper I: Table 1). Light microscopic examination was performed on tissues from necropsies.

2.12. Paper II. Disease complex of Baltic Grey seals, a trend over time study

This material was collected during the 20-year period 1977-1996 when the pollution of the Baltic biota was changing. The DDT concentration in Baltic biota decreased significantly in the beginning of the 1970s and a sudden decrease in PCB concentrations occurred in the period 1975 to 1978 (Olsson and Reutergård 1986, Bignert *et al.* 1995).

One way to detect trends over time as regards the health of the seals was to compare the results between the animals collected during the 10-year-periods 1977-1986 and 1987-1996. During 1977-1986 the concentrations of contaminants were still rather high in Baltic biota while during 1987-1996 these concentrations were decreasing (see Discussion).

Material from a total of 159 grey seals was examined. Necropsy was performed on 145 animals during the 20-year period, 22 females and 21 males in 1977-1986 and 42 females and 60 males 1987-1996 (II: Tables 1-4). In addition, samples of reproductive and non-reproductive organs from 12 adult females were examined (II: Table 5) as well as non-reproductive organs from one subadult and one adult male.

The animal material was divided into the following age classes. Subadults: 1-3-year-olds and adults: 4-15-, 16-25- and >25-year-olds (II: Tables 1-4).

Time at collection will not give a general indication of individual burdens of contaminants. These are also dependent on animal age. The material was therefore also divided according to the year of birth. Two groups, which could be assumed to have different burdens of contaminants, were selected: 1) animals born 1979 and earlier and 2) animals born 1980 and later. With the latter classification only the age class of 4-15 years contained a sufficient number of animals to be evaluated statistically. It comprised five females and eight males born before 1980 and 15 females and 24 males born 1980 and later.

Animals of higher age classes in both the early and late collection periods represent individuals, who have been exposed to high organochlorine concentrations via the food during their lives. Of the two groups of subadults in the two collection periods, one represents individuals with high and one with relatively low exposition for organochlorines.

2.13. Paper III. Skull bone lesions in Baltic grey seals

Paper III concerns skull bones and mandibles of adult (≥6-year-old) grey seals.

A late skull bone material from Baltic grey seals was collected during 1960-1985. During this period the DDT and PCB pollution became severe in the 1960s and reached the up to now highest levels in the beginning of the 1970s (Olsson & Reutergård, 1986). Samples from altogether 193 animals were examined, 68 of which were collected 1960-1969 and 125 during 1971-1985. The material collected 1960-1969 included skulls and mandibles from five and pairs of mandibles (bounty proofs from legal hunting) from 63 animals. The samples from the seals collected 1971-1985 consisted of skulls and mandibles. These animals were found drowned in fishing gears or found dead on the shore. Of the 125 animals in this material, 58 were females, 60 were males while in seven cases sex could not be determined (Paper III: Fig. 4). Sex determinations were only performed in the Baltic material collected 1971-1985.

Age determinations were made according to Johnston & Watt (1981). Otherwise rough age determination was performed by macroscopic investigation of size and morphology of bone and tooth structures.

As references from the Nordic area, skull bones and mandibles from altogether 41 animals, collected before 1950, thus sampled before the increase of organochlorine pollution of the Baltic, were used. Of these were 14 available for studies at the Swedish Museum of Natural History in Stockholm, and 20 and 7 respectively, at the Zoological Museums of Copenhagen and Helsinki.

An additional reference material of 165 skulls was derived from the North Sea area and the North Atlantic area around the British Isles. Of these, 57 were stored at the British Museum of Natural History in London, and 108 at the British Antarctic Survey in Cambridge, UK. Many of the skulls in the British Museum and most of the skulls at the British Antarctic Survey were sampled during the 1960s and 1970s. During the collection period the British area concerned can be considered as being very low contaminated by organochlorines (Donkin *et al.* 1981, Law *et al.* 1989).

2.14. Paper IV. Bone mineral density in Baltic Grey seals

Mandibles and radius bones in male grey seals from Swedish waters were studied. Samples from 43 animals were used. In 28 cases mandibles alone were available, while in the remaining 15 cases, besides mandibles, also radius bones were available for investigation. The material was stored at the Vertebrate Section at SMNH and was collected during three time periods:

1. 1850-1955, a period before the main introduction of organochlorines in the environment. The material comprised mandibles from nine males (9-23-years old): three from the Swedish West Coast and six from the Baltic. There were no radius bones available from this period.
2. 1965-1985, a period of high concentrations of organochlorines in the Baltic biota. Samples from 22 males (4-15-years old) were available: mandibles alone from 17, and mandibles/radius bones from five.
3. 1986-1997, a period of decreasing concentrations of organochlorines in the Baltic biota. Samples from 12 males (4-15-years old) were available: mandibles alone from two, and mandibles/radius bones from 10.

The selection of time intervals was based on results from long term monitoring of concentrations of contaminants in Baltic biota (Olsson and Reutergård, 1986; Bignert *et al.* 1998a.).

The bones were examined by peripheral quantitative computed tomography (pQCT) (Stratec XCT 960A, software version 5.20 Norland Stratec Medizintechnik, Pforzheim, Germany). For geometrical and densitometrical analysis of trabecular bone, peel mode 2, contour mode 1, threshold 0.660 cm -1 and inner threshold 0.700 cm-1 were used. For the same analysis of cortical bone, separation mode 1 and a threshold value of 0.930 cm-1 were used. The bones were placed horizontally and scanned using voxel size C (0.295 mm). Measurements on trabecular area (mm2), trabecular content (mg/mm) trabecular bone mineral density (mg/cm3) total cross-sectional area (including marrow cavity and cortical bone, mm2) cortical area (mm2) cortical content (mg/mm), cortical thickness (mm) and cortical bone mineral density (mg/cm3) were used for the analysis.
The reproducibility of the pQCT measurements was evaluated by calculation of the co-efficient of variation from 10 consecutive measurements with a single sample repositioning before each measurement.

The data were evaluated by one-way ANOVA followed by post-hoc Fisher`s PLSD. Differences were considered significant when $p<0.05$.

Radius

The Radius was scanned at a point distanced 35 % of the length from the proximal part of the bone (IV: Fig. 1). This area was chosen because it is rich in trabecular bone.

Mandibular Bone

The width of the hindmost molar of the left mandibular bone was measured with an accuracy of 0.01mm using an electronic sliding caliper. The subsequent measurement of the bone mineral density was made at a point located twice this width value behind the molar (Paper IV: Fig. 2). This point was chosen because the scanned area contains both trabecular and cortical bone.

2.15. Paper V. Renal lesions in Baltic grey and ringed seals

Light microscopy

Kidneys for light microscopy were from a total of 144 seals from 4 different sites: the Baltic, Swedish zoological gardens, Svalbard and Scottish waters. Samples covering all age groups were examined (Paper V: Table 1). The Baltic samples were from 76 grey seals (36 females and 40 males) and 29 ringed seals (17 females and 12 males). The seals from the Swedish waters were collected in the Baltic and the Gulf of Bothnia during 1977-1996. The oldest Baltic grey seal females aged around 40 years, and the oldest male 23. The grey seal samples from Swedish zoological gardens were from five animals (one female and four males). Of these, the female was 1-year- and the males were 12, 24, 32 and 33-years-old. The zoo-animals had been fed Baltic herring, highly exposed to contaminants, during the major part of their lives (Palm, M. personal communication).

Age determination was performed according to Johnston & Watt (1981). Age classes used were <1, 1-3, 4-10, 11-25 and >25-years old (Paper V: Table 1). To show prevalence of specific lesions in different species, sex and age groups, age classes were restricted to ≤3, 4-25 and >25-year-olds (Paper V: Figures 13-15).

Electron microscopy

The investigation was performed on kidneys of six grey seals, three females and three males. The three females were from the Baltic. Two of them were 35 and one was 40 years old. Of the males, two aged 3 years were from the Baltic and one, aged 33 years was a captive seal. Four seals from the Baltic had drowned in fishing gear. Two seals, one from the Baltic and the captive seal were shot due to serious illness.

Grey seals for comparison were obtained during a hunting expedition to Helmsdale in northern Scotland in 1988. Complete necropsies were made on 11 animals, six females and five males. Three of the females were yearlings and three were subadults while the five males were all subadults (Paper V: Table 1). Ringed seals for comparison were obtained during a hunting expedition to Svalbard in 1981. Kidney samples from 23 Arctic ringed seals: eight females and 15 males were used (Paper V: Table 1). The age distribution of the Arctic ringed seal material (2-32 years) was rather close to that of the Baltic ringed seals (<1-30 years), which were used in this investigation (Paper V: Table 1).

Histological grading

An average of four sections from different sites of the kidneys in each animal was studied by light microscopy.

Three kinds of lesions were graded: glomerular capillary wall thickening (CWT), glomerular capillary wall nodules (CWN) and tubular cell proliferations (TCP).

Classification of glomerular capillary wall thickening was based on two of the authors' experience from domestic animals and man. The lesion was classified into four grades: Zero i.e. no changes, slight, moderate and severe (Paper V: Figs. 1-4). The two authors performed the classification independently. Full agreement was reached in about 90% of the cases. When there was a disagreement, the sections were re-examined together and discussed until agreement was reached.

The prevalence of tubular cell proliferations was calculated using an indexed square in the microscope eyepiece. In sections of each case 100 one-mm2 squares, strictly positioned edge to edge, were investigated at a magnification X100. Seventy such areas were investigated in the renal cortex and 30 in the medulla. For each square, presence or absence of tubular cell proliferations was recorded.

The prevalence of glomerular capillary wall nodules was determined as the percentage of glomeruli with this change in the investigated cortical area (70 mm2).

2.16. Paper VI. Phocine distemper, 1988

During the 1988 Distemper epizootic in seals complete necropsy with microscopic examination was performed on 37 harbour seals and 12 grey seals. The animals were collected from May 19 to the end of November. The harbour seals were collected from the Swedish West Coast (21), Öresund (5) and the Baltic (11). Two of the grey seals were collected in Öresund and ten in the Baltic. Two harbour seals and nine grey seals were found drowned in fishing gear. The remaining 38 animals were found dead at shore or were killed because of signs of disease. Of the harbour seals, 26 were males and 11 were females.

Age determination in grey seals was performed according to Johnston & Watt (1981) while in harbour seals age was estimated as described earlier. The males were two aborted foetuses, seven newborns, eight juvenile-subadults, and nine adults. The female harbour seals were four new-borns, four juvenile-subadults, and three adults. Of the grey seals six were males and six were females. Of the males, two were juvenile-subadults and four were adults. Of the female grey seals, one was juvenile-subadult and five were adults. The group of adults, both harbour seals and grey seals, included some aged individuals.

2.17. Observations during 1997-2002

Necropsies of seals in the SWISP project was continued by the author during 1997-2002. Before 1997 necropsies almost exclusively concerned animals by-caught at fishery or found dead at shore. Thereafter the size of the material is enlarged by samples obtained at a limited legal hunting authorised by the Swedish Environmental Protection Agency. This hunting concerns Baltic grey seals and, to a small extent, harbour seals on the Swedish West Coast. The hunters are obliged to save organ material from the animals for pathologic-anatomic examination and chemical analysis of environmental contaminants. The material from harbour seals collected in this way is mainly used for chemical investigation although female reproductive organs from this species have been examined.

2.17.1. Grey seals

2.17.1.1. Animals found dead in fishing gear or at shore

Yearlings

A material from 44 animals was investigated including whole bodies of 41 (22 females and 19 males) and samples of single or more organs from three (two females and one male). The material was collected during March-December. Twenty-three of the yearlings (12 females and 11 males) were pups, i.e., having persistent embryonic furs. All of them were found dead at grey seal monitoring studies by SMNH at the end of March 1997 (Table 9).

Twenty-one yearlings (12 females and nine males) had passed the pup-stage (Table 9). They were collected during April-December. They are denoted as post pup-stage yearlings in the following. Eighteen of these were by caught at fishery, two were found dead on shore and one, a male, had lost orientation and was found dead at a car park some kilometres from the sea.

Table 9. *Number of pups and post-pup stage Grey seal yearlings by caught at fishery or found dead at shore and collected 1997-2002 subjected to whole-body (WB) and organ sample (OS) investigation.*

Age class	♀♀		♂♂	
	WBI	OSI	WBI	OSI
Pups	12	0	11	0
Post-pup stage yearlings	10	2	8	1
S^n	22	2	19	1

Grey seals, one-year-olds and older

Material from 70 animals (18 females and 52 males) was investigated, comprising whole bodies from 62 cases (12 females and 50 males) and samples of single or more organs from eight cases (six females and two males).

Fifty-three animals (13 females and 40 males) were by caught at fishery, 16 (five females and 11 males) were found dead at shore and one, a male, was killed at shore due to disease.

All animals were age determined by tooth examination according to Johnston & Watt (1981). Fifteen of the 18 females were 1-20; one was 25 (only reproductive organs available for examination), one 32 and one 35 years old. The age-range of the 50 males was 1-18 years. For over-view of the material, see Table 10.

Table 10. *Number of Grey seals, ≥1-year-olds, by caught at fishery or found dead at shore, during 1997-2002 distributed on sex and age (years) subjected to whole-body (WB) and organ sample (OS) examinations*

Age class	♀♀		♂♂	
	WB	OS	WB	OS
1-3	6	3	10	2
4-10	4	0	23	0
11-20	2	0	17	0
>20	0	3	0	0
S^n	12	6	50	2

2.17.1.2. Material obtained via legal and illegal hunting

The examination included material from 23 grey seals, 21 obtained via legal and two via illegal hunting. Whole-body examinations were performed in 14 and organ sample investigations in nine cases. Age was determined according to Johnston & Watt (1981). Seven of the animals were post-pup stage yearlings (six females and one male), four aged 1-3 years (two females and two males), eight aged 4-10 years (three females and five males), and four aged >10 years (one female and three males). Victims of illegal hunting were two males aged 15 and 16 years respectively. The age of the ≥1-year-olds ranged 2-13 years in females and 2-16 years in males. For overview of the material, see Table 11.

Table 11. *Number of grey seals, shot legally or illegally, during 1997-2002 in sex and age classes and subjected to whole-body (WB) and organ sample (OS) examinations*

	♀♀		♂♂	
Age class	WB	OS	WB	OS
Pups	0	0	0	0
Post pup stage yearlings	3	3	1	0
1-3 years	2	0	2	0
4-10 years	0	3	4	1
11-20 years	0	1	2	1
S^n	5	7	9	2

2.17.2. Ringed seals

The material of Baltic ringed seals was small as compared to the grey seals and comprised whole-bodies of 16 animals (eight females and eight males). All of them were by caught at fishery. Age determination by tooth examination is not yet finished in this species; why age classification into yearlings, subadults (1-3-year-old) and adults (≥4-year-old) was done by estimation, as described in Material and Methods.

Of the females, one was a yearling, five were subadults and two were adults, and of the males, five were yearlings and three were subadults.

Table 12. *Number of Baltic ringed seals by caught at fishery, collected during 1997-2002 subjected to whole-body examination, in sex and age classes.*

Age class	♀♀	♂♂
Post-pup stage yearlings	1	5
1-3-year-olds	5	3
≥4-year-olds	2	0
S^n	8	8

2.17.3. Harbour seals

Altogether, material from 11 animals (three females and eight males) was in a condition fresh enough to allow adequate macroscopic examination. Of these, four were found dead on shore, one was found ill on shore and soon died, and six were by caught at fishery.

Age determinations remain to be finished, why age in eight animals was estimated (see 2. 4 Age and age classes). Age determination according to Johnston & Watt (1981) concerned one female: 1-year-old, and two males: 2 and 23 years old. The age estimation showed two female and three male yearlings, one male subadult, and two male adults. Besides the three yearlings ages of three males were estimated to 2, 10 and 15-20 years. Animals of different age classes: yearlings, subadults (1-3-year-olds), and adults (≥4-year-olds) are recorded in Table 13. Whole-body examinations were performed on ten animals and organ sample examination on one (Table 13).

Table 13. *Number of Baltic harbour seals in sex and age classes found dead, killed due to illness or by caught at fishery during 1997-2002, subjected to whole-body (WB) or organ sample (OS) examination.*

Age class	♀♀		♂♂	
	WB	OS	WB	OS
Yearlings	2	0	3	0
1-3-year-olds	1	0	2	0
≥4-year-olds	0	0	2	1
S^n	3		8	

Results, see 3.10.4.

2.17.4. Distemper epizootic 2002

Altogether 30 harbour seals from the Swedish west coast were examined during the course of the epizootic. Twenty-six of these, ten females and 16 males, showed signs characteristic for Distemper (see Paper VI). To verify the spread of the disease the PD diagnosis was set in six animals (three females and three males). The remaining 20 animals with PD were examined during field expeditions and were shot due to severe disease.

Age of 16 of the PD animals was determined according to Johnston and Watt (1980) while in nine animals age was estimated. For age distribution see Table 14.

Table 14. Sex and age classes of harbour seals from the Swedish west coast with distemper, necropsied during the 2002 epizootic.

Age class	♀♀	♂♂
1-3-year-olds	3	4
≥4-year-olds	7	12
S^n	10	16

Four of the ten animals that were found dead on shore (one male and one female foetus, one female yearling and one adult female) were in states of severe autolysis why causes of death could not be confirmed.

2.18. Statistics (Paper II, IV, V and in 'Observations 1997-2002')

Paper II

When possible, due to sample size, Chi-square tests with Yates' correction were applied to study pathological changes over time between grey seals grouped according to the year of birth or to the collection time.

Paper IV.

The data recorded were evaluated by one-way analysis of variance (ANOVA) followed by post-hoc Fisher's PLSD. Differences were considered significant when $p<0.05$. The possible influence by age was investigated by analysis of covariance (ANCOVA).

Paper V

In this report, focused on renal lesions in Baltic grey and ringed seals, statistical tests were performed on results solely from grey seals. The nonparametric Kendall's rank correlation test (1975) was applied. Since repeated Kendall rank correlation tests were carried out on the same material, the α-level was adjusted according to Šidák (1967). The test was used to investigate:

a) The relationship between age and occurrence and grades of three renal histological disease variables: glomerular capillary wall thickening (CWT), glomerular capillary wall nodules (CWN) and tubular cell proliferations (TCP) and occurrence and grades of three specific disease variables recorded in other organs: colonic ulcers (COLU), aortic wall changes (AWC) and adrenal weight (ADW).

b) The relationship between grades of the renal histological variables and grades of the specific organ variables.

c) The interrelationships between the renal histological variables.

d) The correlation between increasing concentrations of heavy metals (Cd, Pb and Hg) in kidney and liver tissues as well as on levels of sPCB and sDDT in the blubber vs. grades of the renal histological variables and grades of the specific organ variables.

e) The relationship between age and tissue concentrations of the chemical compounds under consideration.

Observations 1997-2002

The Mann - Kendall trend test, described in Helsel & Hirsch (1995) was used to investigate correlations between three variables: age, colonic ulcers and adrenocortical hyperplasia in Baltic grey seals aged from post-pup stage, using non-pooled data.

3. RESULTS

3.1. Integuments (Paper I and II)

Regional skin changes occurred in three out of the 13 cases of adult grey seal females that were investigated during 1977-1983 (Paper I). These appeared as more or less advanced hypotrichosis (thin hair coat) localised in the ventral thoracic and abdominal regions with extension to medial surfaces of the fore and hind flippers. In one grey seal female, aged 30 years (Paper I: Table 2) this change also occurred around the supercilia of both eyes. In the latter case, chloracne-like changes were found at microscopic examination, including a thin epidermis, hyperkeratosis and dilation of hair follicles, focally with development of large subepidermal cysts (Paper I: Fig. 1).This kind of lesion has not been observed in Baltic grey seals during this project after 1983. *Lesions in claw regions* were rather common in Baltic grey seals and were graded according to the following characteristics:

1. Slight: occurrence in single claw regions of brittle and upwards bent claws.
2. Moderate: more generalised changes as Grade I (Paper I: Fig. 2).
3. Severe: changes as Grade II, associated with wounds at claw sites (Paper II: Fig. 2).

Microscopic examination revealed unspecific chronic inflammation of claw folds and dyskeratosis of the horn.

The youngest grey seal having a Grade 2-3 lesion was a 5-year-old male, collected in 1993, and the oldest was a female, aged 41 years, and collected in 1986. The prevalence of Grade 2-3 lesions was higher (23%) in the group of 4-15-year-olds born 1979 and earlier (Paper II: Table 6:3) compared with those born 1980 and later (5%) (Paper II: Table 6:4), but the difference was not significant. The prevalence was high in higher age classes, grouped by order of collection (Paper II: Table 6; 5-9), but the number of these animals was too small for statistical evaluation.

Fifteen Baltic ringed seals were investigated during 1977-2002. Two of these showed slight regional skin changes. Lesions of claw regions were observed in four other cases, three of which showed lesions of slight degree, while in one, the lesions were severe.

3.2. Regional intestinal ulcers (Paper I and II)

Ulcers of the intestinal mucosa were a common feature of the BSDC. The ulcers were located in the ileocaeco-colonic region, predominantly in the anterior part of the colon. The changes were always combined with more or less marked hypertrophy of the muscular tunic in the affected region.

Three grades of severity of the lesion were defined according to mucosal extension and depth in the intestinal wall.

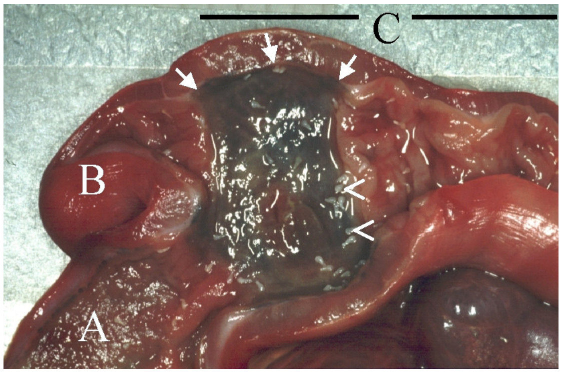
1. Slight: minor mucosal ulceration with a diameter of 3-10 mm.
2. Moderate: mucosal ulceration of areas exceeding 10 mm in diameter, which could extend transversally around the entire intestinal lumen.
3. Severe: large and deep ulcerations (Figs. 5-8) affecting also the muscular tunic often involving the serosa that showed fibrinous or fibrous (Fig. 7) adhesions to adjacent organs. In some cases the ulceration extended through the ileum, caecum and part of the colon (Fig. 8). Fatal ulceration implied open communication to the abdomen (Paper I: Fig. 3, Paper II: Fig. 3).

Mucosal erosions of a pinhead size surrounded by ringed-form aggregations of hookworms constitute the initial lesions. The lesions may be single or multiple and may occur throughout the caecal and colonic mucosa. The intact mucosa has an even margin to the wound and its border is slightly elevated (swollen) within a couple of millimetres close to the wound. The colour of the wound surface is grey, greyish-green or black. In more profound processes some black spots and streaks or white streaks appear in the muscular tunic. In fatal cases, fibrinous and fibrous adhesions usually are found at the perforation site. The content of the colon is often black due to haemorrhages from the wound. As a rule, hookworms are found in or in the vicinity of large wounds (Fig. 5 and 6) but in some cases they may be single or difficult to detect.

According to the author's observations, appearance of hookworms in the intestinal mucosa first occurs during the seal's first summer of life, i.e. when the pup has begun to consume fish. During the first summer and autumn colonic ulcers may occur. As a rule these lesions are slight but there is a tendency towards occurrence of more severe colonic ulcers in yearlings (Fig. 5-6) during recent years. The youngest grey seal found to have a fatal ulcer was a 1-year-old female, collected in 1978, while the oldest, also a female, aged 37 was collected 1979 (Paper I: Table 2). Most perforations were located in the colon a few centimetres posterior to the head of the caecum, opposite to the ileo-colonic orifice (Paper I: Fig. 3, II: Fig. 3). As regards the grey seal material collected 1977-1996, intestinal perforation and peritonitis was, after drowning in fishing gear, the most common cause of death with a prevalence of 7% (11 out of 145 cases, see Paper II: Tables 1-4).

Fatal intestinal ulcers occurred in all age classes, from 1-year-olds (Paper II: Table 7). The highest numbers of grey seals with Grade 2-3 (moderate or severe) colonic ulcers were observed in the groups of 4-15 year old females and males born 1980 and later (Paper II: Table 7; 4) and in animals older than 15 years (Paper II: Table 7; 5-9). The prevalence of Grade 2-3 ulcers was consistently higher in animal groups more recently investigated than in those of earlier periods, but a statistically significant increase was found only in the group of 1-3-year-olds collected 1987-1996 compared with the corresponding age group collected 1977-1986 (Paper II: Table 7; 1-2). Fig. 9 shows a more lucid compilation of the Paper II results on tendency of colonic ulcers in different age classes during 1977-1996.

As regards recent results on this lesion, see below under 'Observations during 1997-2002'.



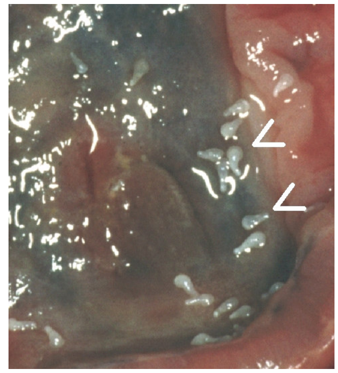


Fig. 5 (left) and 6 (right). Severe colonic ulcer in a grey seal female yearling, found drowned in fishing gear, September 12, 2001. A, ileum. B, caecum. C, colon.The site of the ulcer in the anterior colon (Fig. 5, arrows) is the most common in Baltic grey and ringed seals. In the periphery of the ulcer there are hookworms (arrow-heads), see also magnified area (Fig. 6). Photo: Author/Anna Roos.

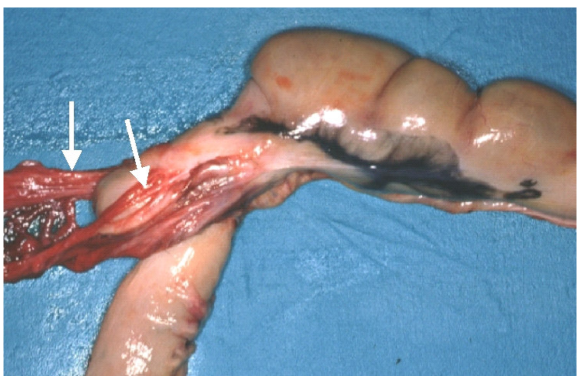
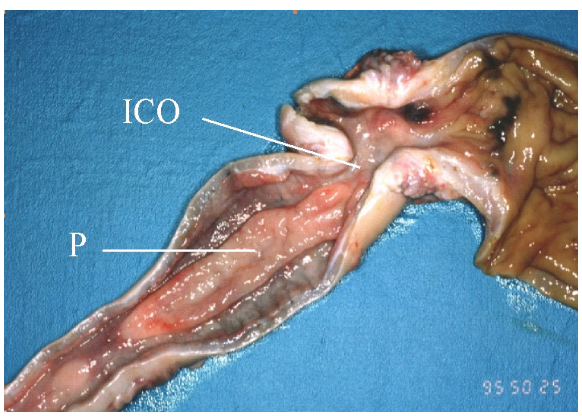



Fig. 7 (above) and 8 (below). Severe colonic ulcer in grey seal female, 30 years old, found dead at shore, March 29, 1995. Fig. 7 shows chronic adherence (arrows) of mesentery to caecum and anterior part of colon and signs of older haemorrhages (black area) beneath the serosa of anterior colon. The cut open aspect (Fig. 8) of the specimen shows a large and deep ulcer with loss of mucous membrane of ileum, caecum and a small part of anterior colon. Note the naked Peyer´s patch (P) of ileum near the ileo-colonic orifice (ICO). Photo: Author/Anna Roos.

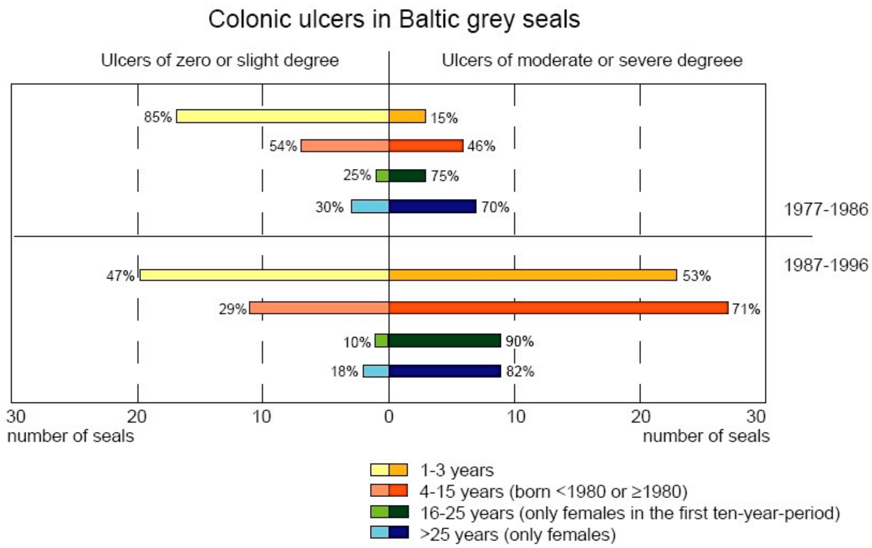


Fig. 9. Graph from records in Paper II. Number and prevalence (%) of grey seals with no or slightly advanced (left) or moderate to severe (right) colonic ulcers, collected during 1977-1996. Three age classes, 1-3, 16-25 and >25-year-olds, are grouped according to period of collection: 1977-1986 and 1987-1996, while 4-15-year-olds are grouped according to year of birth: born before 1980 and born 1980 and later. The prevalence of animals with more severe ulcers is higher in all age classes in latter periods. This increase is significant in 1-3-year-old grey seals. Lay out: Annika Tidlund, Stockholm Marine Research Centre, Stockholm University.

3.3. Vascular lesions (Paper I and II)

The changes in the main arteries were restricted to the aorta and its bifurcations. The changes were most evident in the distal part of the aorta. They were graded as follows:

1. Slight: presence of focal minor whitish intimal spots and streaks with slight intimal thickening.
2. Moderate: larger confluent areas of intimal thickening with sub-intimal lipid deposits.
3. Severe: lesions of the above character occupying most of the vessel and extending around the whole lumen with loss of elasticity.

There were no intimal ulcerations or calcifications. A thrombus was situated at the angles of the pelvic branches in one animal. The prevalence and severity of vascular lesions were significantly correlated with advanced age. Changes of Grade 2-3 were most common in elderly females collected both 1977-1986 and 1987-1996. (Paper II, Table 8: 8-9).

3.4. Adrenocortical hyperplasia (Paper I, II and V)

The degree of hyperplasia was based on the macroscopic appearance at necropsy (Paper I and II). It was exclusively located in the cortex (I: Fig. 4, and II: Fig. 4). Four grades (zero, slight, moderate and severe) of adrenocortical hyperplasia were defined according to the degree of thickening (Paper II: Fig.4). Adenomas (II: Fig.4) were frequent in the enlarged glands. In the first investigation all grey seal and ringed seal females aged ≥10 years showed some degree of cortical hyperplasia (I: Table 2).

All grey seals in age classes >15 years old in the second investigation showed adrenocortical hyperplasia of at least Grade 2 (moderate) (Paper II, Table 9). There were no significant differences between the prevalence of this change in early and late collection or birth periods (Paper II, Table 9).

In paper V, instead of cortical thickening, total weight was used as the adrenal variable in the statistics on age and inter-organ-change.

3.5. Lesions of female reproductive organs in Baltic grey-and ringed seals (Paper I and II)

These lesions regard uterine occlusions/stenoses and uterine tumours (leiomyomas) the prevalence of which as well as the rate of pregnancy was determined in sexually mature (≥4 years) females.

3.5.1. Stenoses and occlusions

Fig. 10 shows a normal pregnancy in a Baltic grey seal. The stenosis (narrowing) and the occlusion of the uterine lumen are most often localised in the middle part of the uterine horn (Fig. 11). At the site of the obstruction the uterine wall usually is thickened. In the grey seal, the obstructions usually are less developed, with the character of stenosis (Paper II: Fig. 5), than in ringed seals in which obstructions of occlusion-type (Fig. 11) predominate. Both these kinds of obstruction cause accumulation of fluid, as a rule of a serous character (Paper I: Fig. 6) with distension of the uterine lumen and flattening of the mucosal folds of the anterior part of the affected horn (Paper II: Fig. 5 and Fig. 19, this chapter). The youngest grey seal female found to have occlusion of the uterus was 7 years old and was necropsied in 1985 (Paper II). The same age for the first occurrence of occlusion is also reported for Baltic ringed seals (Helle, personal comm.). Three grey seal cases described in Paper I showed retained foetal membranes aligned to stenosed parts of the uterine horns, as sign of abortion. Besides, the uterus of two of these cases also showed decayed foetuses, in one case concomitant with a purulent endometritis (Paper I: Fig. 5).
The material of reproductive organs from female Baltic ringed seals and grey seals in the first investigation of animals collected 1977-1983 (Paper I) consisted of organ samples (I: Table 1) and material obtained at necropsies (I: Table 2). When adding the records in these Tables, 18 out of 27 sexually mature Baltic ringed seals (67%) had uterine obstructions (occlusions or stenoses). For grey seals the

corresponding figures for prevalence of occlusion/stenosis was 19 out of 66 (29%) and for uterine tumours (leiomyomas) 19 out of 68 (28%).

Animals collected 1977-1983 were included in the trend over time investigation 1977-1996 (Paper II), which exclusively concerned female grey seals. Occlusion/stenosis of the uterus was found in eight out of 19 cases (42%) from the early collection period 1977-1986, while the corresponding figures for the late collection period 1987-1996 were four out of 37 (11%). This means a statistically significant decrease of these lesions in the late collection period ($p < 0.05$, II: Table 10) compared with that of the early period.

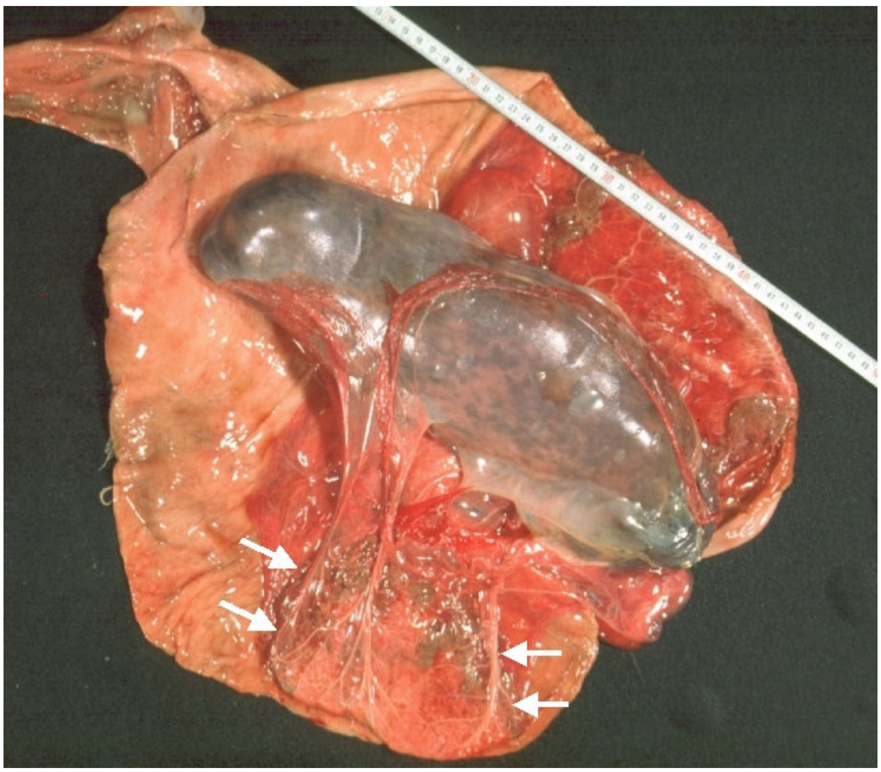


Fig. 10. Pregnancy in Baltic grey seal, showing the zonary (girdle) type of placenta (within arrows). The foetus, enclosed by the amnion, is a male (41 cm, 1.38 kg). The 20-year-old mother was caught in fishing gear on October 31, 1982. Photo: Bengt Ekberg, National Veterinary Institute, Uppsala.

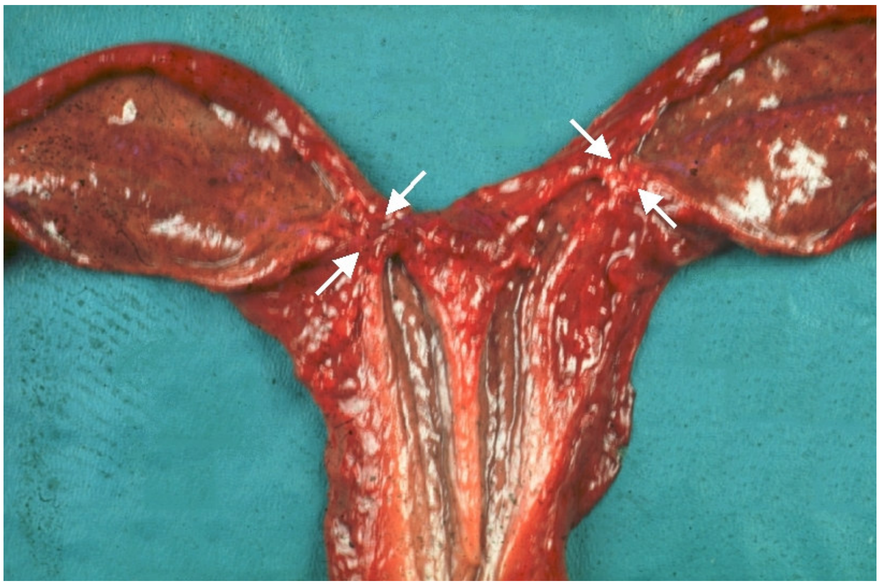


Fig. 11. Occlusion (arrows) of both uterine horns in a Baltic ringed seal, 28-30 years old, collected on October 8, 1978. Note dilated anterior parts of the horns with flattening of folds of the mucous membrane due to fluid accumulation. Photo: Mats Olsson, SMNH.

3.5.2. Leiomyomas

A large prevalence of uterine leiomyoma was observed in Baltic grey seals but no case in Baltic ringed seals. The tumours show the same characteristic gross appearance. They are of firm consistency, white-brown to white-yellow on cut surfaces, which show a whirled pattern, as occurring in leiomyoma and they were localised preferably in the wall of the uterine corpus (Paper I: Fig. 6, Paper II: Fig. 6). They were often multiple and might be large (Paper I: Fig. 6), up to 10 centimetres in diameter. Occasionally, large tumours showed central areas of necrosis. For 21 out of 26 cases collected 1977-1997, the tumours were confirmed histologically to be leiomyoma, while in the remaining five cases, comprising samples of reproductive organs, the diagnosis of leiomyoma was based upon the macroscopic appearance. In Baltic grey seals the uterine leiomyoma usually appear at the age around 30 but they were also found in two females, aged 15 and 22 years, autopsied in 1995 and 1993 respectively.

The number of grey seal females with leiomyomas recorded in the early collection period, 1977-1986, was 10 out of 19 (53%) and during the late period, 1987-1996, 16 out of 37 (43 %). This decrease in prevalence during the late compared with the early period was not significant statistically (II: Table 10).

3.5.3. Pregnancies

Determination of pregnancy in grey seals was performed within the seasons of so called active gestation (see Introduction), from mid August up to the period of births, during the 20-year period of investigation, 1977-1996.

One of 11 (9%) of the females in the early collection period, 1977-1986 and 12 out of 20 (60%) in the late collection period, 1987-1996, were pregnant. This difference was statistically significant ($P<0.05$). The collection dates for these females were from September 9 to and including November 3.

When comparing the prevalence of the findings recorded in the female reproductive organs of Baltic grey seals during 1977-1986 with those of 1987-1996 (Paper II: Table 10), all indicate improved gynaecological health. A significant decrease of uterine occlusions/stenoses and an increased frequency of pregnancies were recorded in the recent compared to the earlier period. A positive tendency, although not significant, also regards the prevalence of uterine leiomyomas. For an overview of these tendencies, see Fig. 12.

It should be mentioned that there were no pathologic changes in the female reproductive organs of the Arctic ringed seals used as references in Paper V.

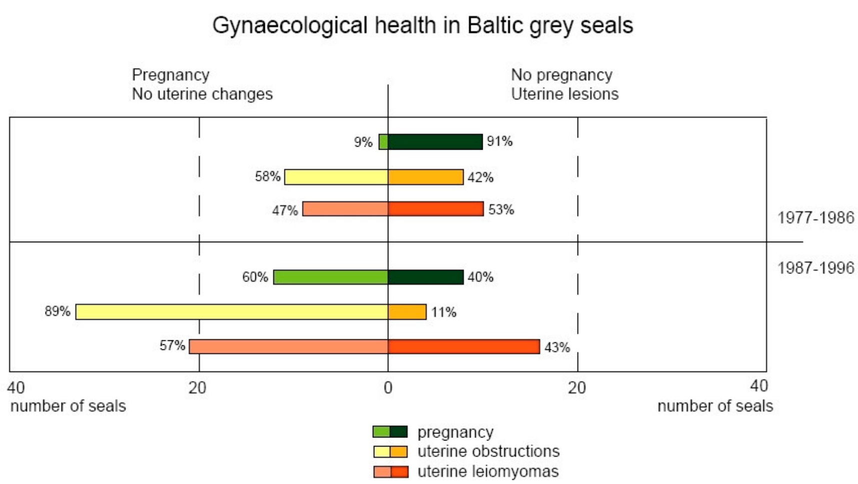


Fig. 12. Gynaecological health in sexually mature (≥4 years old) Baltic grey seals, during two ten-year periods. Pregnancy is determined from mid August to first week of February inclusive. The prevalence of pregnant females is significantly higher, and the prevalence of females with uterine obstructions is significantly lower during the later than in the earlier period. There is a tendency towards less prevalence of uterine leiomyomas in the later period but this change is not significant. Layout: Annika Tidlund, Stockholm Marine Research Centre, Stockholm University.

3.6. Skull-bone lesions in Baltic grey seals (Paper III)

Lesions were most often found around the canine teeth and in the incisive part of the masticatory bones, but also around the premolars and molars both in the upper and lower jaws. In mild cases - Grade I or early lesions, loss of bone was restricted to the periodontal lamella with widening of the sockets of the teeth (Paper III: Fig. 1). The teeth often lacked their bony support and had been lost. In severe cases (Grade II lesion), there was a substantial loss of bone especially of the incisive part, in some cases in connection with bone apposition and deformation comprising all parts of the jawbones (Paper III: Fig. 2).

The prevalence of skull bone lesions was high both in Baltic samples from the 1960s and from 1971-1985. As seen in Paper III: Fig. 3, the prevalence was between 30 and 50 % during the 1960s and between 45 and 65 % during 1971-1985. The prevalence was lower, less than 20%, in seals from the Baltic sampled before 1950, while a still lower prevalence, less than 10%, of the same kind of lesions was found in the British material (Paper III: Fig. 3).

The lesions were age related. The highest prevalence, 85 % was found among male Baltic grey seals in the age group 21-30 years, collected 1971-1985, while the prevalence among females of the same age was 50 % (Paper III: Fig. 4). Severe lesions dominated in these age groups. An unexpected finding in Baltic seals was that severe lesions were present also in the age group of 6-10 years born 1979 and earlier, as well as in subadult (1-3 year-old) animals collected 1971-1985.

3.7. Bone mineral density in Baltic grey seals (Paper IV)

The trabecular bone mineral density of the radius was significantly higher in bones collected 1986-1997 than in those collected 1965-1985 ($p<0.05$). The cortical bone mineral density of the mandible was highest in specimens collected 1850-1955 and showed a continuous decline in specimens during the two following collection periods 1965-1985 and 1986-1997 ($p<.05$). (Paper IV: Tables 2-4).

3.8. Renal lesions in Baltic grey- and ringed seal females (Paper I) and in Baltic grey seals of both sexes (Paper V)

Renal lesions described in Paper I are included in Paper V.

3.8.1. Macroscopic appearance and comparative light microscopy

Kidneys with severe lesions were pale, hard and had a lustreless cut surface. There were no scars or change of shape in any of the animals. One male grey seal showed signs of embolic nephritis with several abscesses. One male ringed seal had a ramified calculus in the renal pelvis.

There were no differences between grey and ringed seals as regards the character of the light microscopic changes. They are therefore treated together in the following.

3.8.2. Glomerular lesions

Light microscopy

Glomerular capillary wall thickening (CWT)

The most prominent lesion was a diffuse thickening of the capillary basement membranes (Paper V: Fig. 2-4). No proliferation of capillary endothelial or epithelial cells could be observed at this level of resolution except occasional epithelial proliferations on the inside of Bowman's capsule (crescents) in advanced lesions. The basement membranes stained brightly with haematoxylin &-eosin. Silver staining showed no deposits or duplications of the basement membranes. In severe cases a slight increase in mesangial width and matrix with slight hypercellullarity was present.

The basement membrane of Bowman's capsule was less frequently thickened and laminated. Segmental sclerosis with adhesions between capillaries and capsule was observed in a few cases. Total global sclerosis was seen in a varying number of glomeruli in connection with vascular and interstitial lesions. Small deposits of calcium were seen in glomerular capillary walls of grey seals with severe changes.

Electron microscopy

Electron microscopy verified the thickening of the glomerular capillary basement membranes in five animals but autolysis did not permit further observations. The material from the 40-year-old female grey seal, which was shot, was well preserved. In this case the glomerular basement membrane was duplicated with an expansion of mesangial cells into the membrane (mesangial interposition, Paper V: Fig. 8-10). The capillary epithelial cells were swollen with a slight fusion of foot processes. The endothelial cells were also swollen and dislocated from the basement proper. There were no electron dense deposits on the epithelial or endothelial side of the basement membrane.

Glomerular capillary wall nodules (CWN)

A notable feature observed in several cases was large hyaline nodules in the capillary walls (Paper V: Fig. 5) or in the basement membrane of Bowman's capsule. They were structure-less, stained pale red with H & E and bright red with PAS but not with silver stains. They were negative with methods for fibrin or amyloid. In a few grey seals similar nodules were observed in arteriolar walls at the glomerular hilus.

Amyloid (Paper V: Fig. 6) was present in the glomeruli of one animal, a 17-year-old male with several infected shot wounds. Staining for neutral fat showed small droplets occasionally congregated to larger globules in the capillary epithelial cells in 15 grey seals, 13 females and two males, in connection with fatty changes in the tubular epithelium.

3.8.3. Tubular lesions

Light microscopy

Tubular cell proliferations (TCP)

A prominent feature was intraluminal proliferations of epithelial cells in the distal convoluted tubules and collecting ducts (Paper V: Fig. 5 and 7). The cells were monomorphic, large, pale and polygonal. In some cases they consisted of a monolayer on the tubular basement membrane, in others the tubules were distended and completely filled with solid islands of cells. They were always strictly located inside the basement membrane. Fat staining revealed fatty droplets in the cell cytoplasm mainly in the proximal convoluted tubules and in the medulla in connection with old age and severe glomerular and interstitial changes. This fatty change was focally distributed, usually in the form of fine droplets. In one case only, having pronounced accumulation of lipids in the proximal convoluted tubules, lipids also occurred in proliferated cells as fine droplets, exclusively localised in the periphery of the cell clusters, close to the tubular basement membrane.

Proteinaceous casts were present above all in connection with proliferative changes in the tubular epithelium to be described below. Hyalinisation of the tubular basement membrane was a common finding in connection with interstitial fibrosis. Small calcium deposits were observed, above all in the tubular basement membranes or epithelial cells.

Electron microscopy

Tubular cell proliferations (TCP) could be studied only in the 40-year-old Baltic grey seal female. At high magnification (Paper V: Fig. 11) individual cells of the proliferations were found to be cylindrical or polygonal with a large elongated nucleus. The chromatin was sparse and membrane-associated. The nucleoli were large. The cytoplasm contained, above all in the superficial parts of the cells, a great number of small vacuoles without content. There were shallow invaginations of the basal cell membrane. The intercellular spaces were narrow but frequently dilated containing interdigitating finger-like protrusions (Paper V: Fig. 12). Tight junctions were present at the surface and desmosomes were numerous. Cell organelles were few. There were very few mitochondriae and a sparse endoplasmic reticulum but a great number of free ribosomes. In many cells, clusters of large, weakly stained osmiophilic bodies limited by a single membrane were seen close to the nucleus.

3.8.4. Age, sex and species differences of renal changes

A survey of the prevalence of specific renal changes in the different materials according to age, sex and species is presented in Paper V: Fig. 13-15. The scores for CWN and TCP calculated as described above have been divided to obtain the same four grades of changes as for CWT as follows. For CWN: zero no changes, slight 1-20%, moderate 21-40% and severe >40%, the highest score being 59%. For TCP: zero no changes, slight 1-25, moderate 25-50 and severe >50, the highest score being 78.

A comparison regarding frequency and grades of histological variables between the sexes was performed using only two age classes (≤3 years and 4-25 years) since there were too few animals aged 11-25 years.

Slight TCP were found in two out of 12 females, and in nine out of 24 males among Baltic grey seals, aged ≤3 years and one of the males showed slight CWN (Paper V, Fig. 13 A and B). There were no changes among the Scottish grey seals of corresponding age.

All three types of the renal changes under consideration were seen both in Baltic grey seal males and females, aged 4-25 years. There was a higher prevalence of CWT and CWN in females than in males, but the opposite relationship was found as regards TCP (Paper V, Fig. 14A and B). Most extensive and severe changes were seen in the 15 female Baltic grey seals aged >25 years (Paper V, Fig. 14C).

Compared with the findings in grey seals slight and less frequent changes were present among Baltic ringed seals of both sexes (Paper V, Fig. 15A). These animals were aged ≤ 1-30 years showing a similar age distribution as the Arctic ringed seals investigated: 2-32 years. The latter showed no signs of the specific renal lesions under consideration (Paper V, Fig. 15B).

3.8.5. Statistics on renal lesions, lesions of other organs and age in Baltic grey seals

Grades of specific renal histological changes (CWT, CWN and TCP), adrenal weight (ADW) and aortic wall changes (AWC) were significantly correlated with age in both sexes except for CWN in males. Colonic ulcers (COLU) were not correlated with age (Paper V: Table 2).

There was a significant correlation between adrenal weight and the renal lesions in both sexes except for CWN in males. Colonic ulcers were significantly correlated with CWN and TCP in both sexes and with CWT in females. Aortic wall changes showed significant correlation with the renal lesions (Paper V: Table 3). The three renal histological variables investigated were significantly correlated with each other except for CWN and TCP in males (Paper V: Table 3).

3.8.6. Chemical analyses

Heavy metals

There was no significant positive correlation between concentrations of cadmium, lead and mercury and increasing age, nor there was significant correlation between levels of these heavy metals and grades of the six pathological changes mentioned above (Paper V: Table 5).

DDT and PCB

Blomkvist *et al.* (1992) reported that the young grey seals from Scotland, described in the chapter “Reference animals” (Ch. 2.3), had much lower body burdens of these organochlorines than the Baltic grey seals of the corresponding age (Blomkvist et. al., 1992). Data from the present investigation (Paper V) showed that there was a significant positive correlation between concentrations of sDDT and sPCB and age in Baltic grey seal females ($p<0.01$ and $p<0.001$ respectively) but not in males. The females showed a significant positive correlation between increasing concentrations of sPCB and sDDT and 2 types of renal lesions: CWT ($p<0.01$ for sPCB and $p<0.001$ for sDDT) and CWN ($p<0.01$ and $p<0.01$ respectively). Females also showed a significant positive correlation between increasing concentrations of PCBs and aortic wall changes ($p<0.01$). In males, increasing concentrations of sDDT showed a significant negative correlation with colonic ulcers ($p<0.001$). There was no further significant correlation between the levels of these organochlorines and the grades of pathologic changes.

3.9. Distemper epizootic in 1988 (Paper VI)

In April 1988 the first signs of a severe epizootic among harbour seals appeared at the isle of Anholt in Kattegat (see Fig. 25, map). It spread rapidly throughout the waters of northwestern Europe along the coast of Schleswig-Holstein and the Netherlands and the Swedish West Coast in May. The disease reached colonies of harbour seals in the southern Baltic about one month later. The total mortality until November 1988 was estimated to be at least 17 000 animals with a mortality rate of about 60 % in Danish-Swedish waters (Dietz *et al.* 1989). Besides harbour seals, also a limited number of grey seals from Swedish waters died from the disease.

The diagnosis of a canine distemper-like disease (Osterhaus & Wedder, 1988) in the seals was the result of findings by Swedish pathologists and Dutch virologists. In the Swedish investigation changes similar to those present in canine distemper were found in the upper and lower respiratory tracts, lower urinary tract and the lymphatic system (Paper VI). The Swedish and Dutch results were presented at press conferences in Sweden and the Netherlands, 29 August 1988. Cosby *et al.* (1988) tentatively named the pathogen phocine distemper virus (PDV) and found its antigenic properties to be more closely related to canine distemper virus (CDV) than to other morbilli viruses. Diseases in seals caused by CDV or other morbilli viruses had not been reported previously.

3.9.1 Necropsy

Thirty-seven harbour seals (26 males and 11 females) and 12 grey seals (six females and six males) were investigated during the epizootic. Twenty-one harbour seals were from the Swedish west coast, five from Öresund and 11 from the Baltic. Two grey seals were from Öresund and ten from the Baltic. Thirty-eight of the 49 seals were found dead on shore or were killed due to disease and 11 were by caught at fishery.

Age was estimated as described in Material and Methods. The harbour seals were two aborted male foetuses, seven male and four female newborns, eight male and four female subadults, and nine male and three female adults. The grey seals were two male and one female subadult, and four male and one female adult.

Signs of distemper were evident in the respiratory tract and the lymphatic organs. Inflammatory changes of the lungs were consistent findings in affected animals. A lesion secondary to the pneumonia was severe emphysema of the lungs, mediastinum and the subcutaneous tissues of the dorsal parts of the neck and thoracic regions. The lymphatic organs were smaller than normal, except for the bronchial lymph nodes, which were enlarged. Tissue of the thymus, which is voluminous in young seals and easily observed in seals up to 15 years old, was difficult to detect.
Two out of 13 aborted or full-term harbour seals showed lesions characteristic for distemper. The remaining 22 harbour seals and three elderly (>20 years) females, the two from Öresund and one from the southern Baltic showed more or less pronounced changes of the disease.

3.9.2 Light microscopy

Respiratory tract
The predominant lesion of the lungs was an interstitial pneumonia showing widened interalveolar septa due to oedema and infiltration of mononuclear cells, mainly histiocytes. Single granulocytes were also seen. The alveoli were distended but usually empty. Bronchopneumonia with exudate in the lumen and abscesses was also observed in several animals. In such animals the trachea was the site of catarrhal inflammation. Eosinophilic intracytoplasmic inclusion bodies were often observed in the epithelial cells of the trachea (Paper VI: Fig. 1 and 2.). Special staining for inclusion bodies, see Fig. 13.

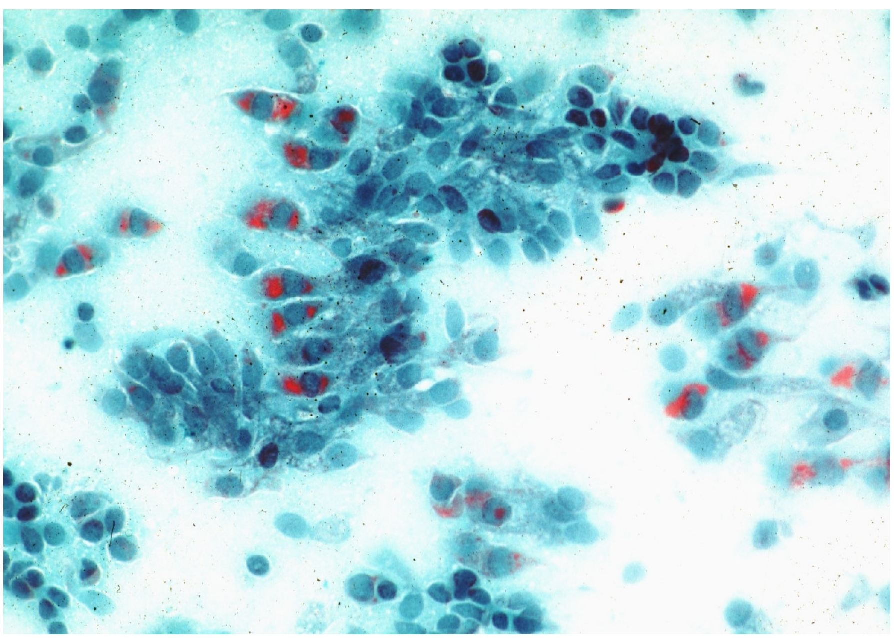


Fig. 13. *Smear from tracheal epithelium of a grey seal female from the southern Baltic, victim of the distemper epizootic in 1988. Red-stained intracytoplasmic inclusion bodies are present in the epithelial cells. Shorr, and Page/Green technique. Author's preparation.*

Lymphatic organs
In the thymus, a general depletion of lymphocytes was the most prominent change. Cortical areas were most severely affected by depletion of lymphocytes and appeared thinner than normal with indistinct medullary borders. The medullary areas were less affected, which led to an inverse relationship between cortex and medulla in respect of cellullarity, cortical inversion (Järplid, 1968). In some animals only a few scattered lymphocytes remained in the cortex. A rim of cells with an immature appearance sometimes appeared just inside the capsule. The medulla was also occasionally atrophic, depleted of lymphocytes and having degenerated and partially calcified Hassall's corpuscles (Paper VI: Fig. 4).

Lymphocyte depletion in the lymph nodes led to atrophy of the cortex and paracortical regions with no active follicles (Paper VI: Fig. 6). The sinuses were dilated and filled with macrophages and other mononuclear cells (histiocytosis).

In the spleen the most prominent lesion was located in the white pulp. The lymphocytes both of the thymus dependent periarteriolar lymphocyte sheath (PALS) and the thymus independent lymphoid follicles were depleted (Paper VI: Fig. 8). There was hyperaemia in the red pulp and histiocytosis with accumulations of macrophages.

Tonsils and gut associated lymphatic tissue (GALT) were also severely depleted of lymphocytes (Paper VI: Fig. 9).

Alongside with signs of distemper some adult grey seals showed lesions of the same kind as in the BSDC.

The lesions in the respiratory organs with interstitial inflammation and a pure exudation of mononuclear cells are characteristic of a virus-induced disease. The same applies to the presence of inclusion bodies in the tracheal epithelium. The depletion of both T- and B- cell areas are probably an effect of a lymphocytolytic effect of the virus (Tizard, 1987).

There were no signs of an acute phase of canine distemper inflammation in the lungs e.g. accumulation of syncytial cells in the alveoli. Neither were there any signs of an acute destruction of lymphocytes. The presence of purulent pneumonia, which was most probably due to a secondary bacterial infection, indicates that the disease had persisted for some time. Signs of lymphatic tissue regeneration were seen only in occasional animals with a rim of immature cells in the thymic cortex.

3.10. Observations during 1997-2002

The report on the results is focused on lesions of the BSDC with especial emphasis on colonic and adrenal lesions. The lesions were graded as described in Material and Methods: zero, slight, moderate and severe changes (Grade 0 to 3). In Tables 14 and 15 animals having grade 0 and 1 are pooled to one group and animals having grade 2 and 3 pooled to another group of the intestinal and adrenal changes.

3.10.1. Baltic grey seals

The results from macroscopic examination of material from animals by caught at fishery or found dead at shore are presented separately from those obtained from animals killed at hunting.
A relatively small number (13 cases) of sexually mature grey seal females were examined during 1997-2002. The findings in female reproductive organs are therefore treated together below under this heading, regardless of the way of collection.

3.10.1.1. Grey seals found dead in fishing gear or on shore

Pups
Twenty-one (11 females and ten males) of the 23 pups were all found dead on shore and collected at seal monitoring studies in 1997. The animals were born on skerries during the unusually mild winter and spring 1997. The mothers lacked access to fast ice and furthermore, they had difficulties to reach the pups for nursing during a period of prolonged stormy weather. All of them were subjected to whole-body examinations (Ch. 2.17.1, Table 9). No one showed signs of lesions belonging to the BSD complex.

The pups showed the following causes of death:

- dehydration-emaciation: 14
- drowning in skerry water pools: 2
- omphalitis-emaciation: 1
- pneumonia: 1
- peritonitis-pleuritis: 1
- traumatic lesions: 1
- cause not confirmed: 1

The two remaining pups, a male and a female, collected in the beginning of April 1997 and 2000, respectively, had drowned in fishing gear.

Post-pup stage yearlings
Eighteen (ten females and eight males) of the 21 post-pup stage yearlings (12 females and nine males) were subjected to whole-body examinations (Ch. 2.17.1, Table 9).

Intestinal (colonic) ulcers
The intestine of 19 animals and adrenals of 17 animals could be examined. Pooled data on these lesions are presented in Table 15. Five of the animals showed colonic ulcers of moderate degree. The earliest appearance of a slight colonic ulcer was in a female, collected in the beginning of June and that of a moderate degree in a male, collected in the end of July. A severe colonic ulcer was observed in a female, who was the only yearling with adrenal change: a slight adrenocortical hyperplasia. The colonic ulcer of this animal, collected on September 12, 2001, is shown in Figs. 5-6.

Other organs

Integument (skin and claw regions), arteries, reproductive organs and skull bones showed no lesions.

Causes of death

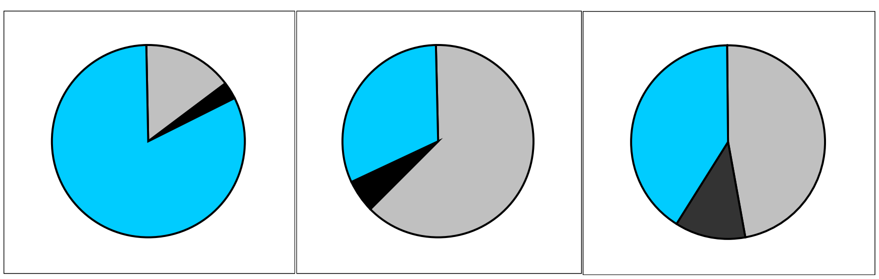
As said earlier (Ch. 2.17.1) 18 animals were by-caught at fishery. The male post-pup yearling, found dead far from the sea, had died from emaciation. The two remaining animals cause of death was not confirmed: they were partly consumed by scavengers.

Grey seals, one-year-olds and older

Colonic and adrenal changes

As for the post-pup stage yearlings, pooled data on colonic ulcers and adrenocortical hyperplasia from the ≥1-year-old grey seals are shown in Table 15. Moderate to severe and fatal colonic lesions evidently predominate in males (eight fatal cases) with an increasing incidence with age. The youngest male with a fatal colonic ulcer was 2 years old.

Adrenocortical hyperplasia occurs later than colonic ulcers but otherwise, the prevalence and severity of this lesion agree well with those of colonic ulcers.
In Fig. 14 the records on colonic ulcers of the 68 grey seals, 14 females and 54 males, aged 1-20 years and listed in Table 4, are compared with the corresponding findings in intestines of female and male grey seals of the same age-range sampled in the same way during 1977-1986 and 1987-1996.



1977-1986	1987-1996	1997-2002
n=33	n=87	n=68
13 females, 20 males	29 females, 58 males	14 females, 54 males

Fig. 14. Proportion of no or slightly advanced (blue), moderate to severe (grey) and fatal (black) colonic ulcers in Baltic grey seal females and males, 1-20 years old, in three consecutive periods. The animals are by-catch at fishery or found dead at shore. There is a higher prevalence of more severe colonic ulcers in the later part of the period of investigation, 1977-2002.

Table 15. *Colonic ulcers (COLU) and adrenocortical hyperplasia (ACH) in Baltic grey seals obtained as fishery by-catch or found dead at shore 1997-2002. Both kinds of lesions were investigated in 83 of the 89 cases. Sex and age class distribution of cases with zero or slight (Grade 0-1) and moderate or severe (Grade 2-3) lesions are shown. Numbers of cases with fatal colonic ulcers are set within brackets. The yearlings have passed the pup-stage.*

		Females			Males		
Age, years		n, cases examined	Grade 0-1	Grade 2-3	n, cases examined	Grade 0-1	Grade 2-3
<1	COLU	13	8	5	6	5	1 (0)
	ACH	11	11	0	6	6	0
1-3	COLU	8	4	4	13	7	6 (1)
	ACH	8	8	0	12	11	1
4-10	COLU	4	2	2	24	10	14 (3)
	ACH	4	4	0	22	13	8
11-20	COLU	2	2	0	17	3	14 (4)
	ACH	2	1	1	16	0	16
>20	COLU	2	1	1	0	0	0
	ACH						
S^n	COLU	29	17	12	60	25	35 (8)
	ACH	27	25	2	56	30	26

Arterial lesions

Large arteries from ten females, aged 2 to 20 years, and from 47 males, aged 1 to 18 years, were available for investigation. Only a 20-year-old female showed arterial lesions and the change was of a mild degree. Large arteries from the two females aged >30 years were not available for investigation. There were no such lesions in males younger than 9 years (15 animals). Of the 32 males aged >8 years, no changes were observed in 17, slight in 11 and moderate in four. Of the males with moderately advanced arteriosclerosis one was 12-, one was 15- and two were 18-years old.

As for colonic ulcers and adrenocortical hyperplasia the incidence of arterial lesions is high in males.

Other lesions of the BSDC

Grade 2 lesions of claw regions were found in the 35-year-old female. There were no other lesions of the BSDC observed, however, part of the skull bone material remains to be investigated.

Female reproductive organs

Results from the investigation of these organs are accounted together with those of the animals obtained by hunting (see below).

Causes of death

Most of the 55 animals obtained via fishery by-catch were drowned and a few were killed in fishing gear.

Of the four females found dead at shore, whole body investigation on one, aged 5 years revealed a severe colonic ulcer and a large liver abscess. For the remaining three animals, aged 25, 32 and 35 years, the material was limited to organ samples, why cause of death was not confirmed. The 35-year-old female showed a severe colonic ulcer, which probably had contributed to death. This animal also showed small tumour-like colonic lesions.

Whole body investigations were performed in all the 11 males found dead at shore. As mentioned above, eight males had died from severe colonic ulcers (see Table 15), seven of them due to colonic wall perforations and one from a severe ulcer complicated by a volvulus of the mesentery. Of the remaining three animals, one, aged 12 years, died from an invagination of the oesophagus into the stomach and one from peritonitis and liver lesions while, in the remaining animal cause of death was not confirmed – part of the carcass was consumed by scavengers.

3.10.1.2. Grey seal material obtained via legal and illegal hunting

Colonic and adrenal changes

Pooled data on colonic ulcers and adrenocortical hyperplasia are given in Table 16. In 21 of the 24 animals both adrenals and intestines were available for investigation. Although the material is small, the same tendency of prevalence and severity of these lesions as in the material from animals found dead at shore or obtained as fishery by-catch (Table 15) can be seen in Table 16. The non-pooled data on these lesions are used in the statistics below.

Table 16. *Colonic ulcers (COLU) and adrenocortical hyperplasia (ACH) in Baltic grey seals obtained at hunting 1997-2002. Both kinds of lesions were investigated in 21 of the 24 cases. Sex and age class distribution of cases with zero or slight (Grade 0-1) and moderate or severe (Grade 2-3) lesions are shown. The yearlings have passed the pup-stage.*

		Females			Males		
Age, years		N, cases examined	Grade 0-1	Grade 2-3	n, cases examined	Grade 0-1	Grade 2-3
<1	COLU	6	6	0	1	1	0
	ACH	5	5	0	1	1	0
1-3	COLU	2	1	1	2	1	1
	ACH	2	2	0	2	2	0
4-10	COLU	3	2	1	5	3	2
	ACH	2	2	0	5	1	4
11-20	COLU	1	1	0	4	0	4
	ACH	0	0	0	4	0	4
S^n	COLU	12	10	2	12	5	7
	ACH	9	9	0	12	4	8

Other lesions
There were no other changes observed of the BSDC but arterial lesions, which occurred in the three oldest males. This was slight in those aged 14 and 15 years, and moderate in the 16-year-old one.

Female reproductive organs
Reproductive organs from 13 sexually mature grey seal females were examined. Nine of them were by caught at fishery or found dead at shore, and four were obtained via hunting. Age, date and way of collection, pregnancy, and presence of uterine tumours in these animals are recorded in Table 17, below. None of the females showed uterine stenoses or occlusions.

Table 17. *Pregnancy and uterine leiomyoma in sexually mature Baltic grey seals investigated during 1997-2002. The cases are ordered according to day and month of collection. Cases no. 1-10 are sampled during a period in which pregnancy can be determined with certainty. FD, found dead at shore. BCF, by-catch at fishery. +, –, Presence or not of pregnancy and leiomyoma. 1) Signs of abortion. 2) Signs of foetal resorbtion.*

Case no.	Age, years	Date of collection	Way of collection	Pregnancy	Uterine leiomyoma
1	25	1997-08-28	FD	–	–
2	12	2000-09-18	BCF	+	–
3	7	2000-09-28	BCF	+	–
4	8	2001-10-04	Hunting	+	–
5	5	1998-10-09	BCF	–	–
6	7	1997-10-29	BCF	+	–
7	13	2001-11-01	Hunting	+	–
8	32	2000-12-04	FD	–[1]	+
9	5	2001-12-06	Hunting	–[2]	–
10	20	2002-02-08	BCF	+	–
11	35	1997-02-23	FD	–	+
12	6	2002-05-09	Hunting	–	–
13	5	2002-08-06	FD	–	–

Cases 1-10 in Table 17 are sampled during a period when pregnancy in Baltic grey seals can be confirmed with a fair amount of certainty (see Paper II). Six of cases 1-10 were pregnant. There is no information on cases no.1 and no. 8 about other lesions and causes of death. Case no. 13 is the female that died from a severe colonic ulcer and a large liver abscess.

The material is not large but there is a tendency, like in the investigation described in Paper II, towards a normal reproductive outcome in the population. The 32-year-old female (case no. 8) showed signs of abortion that is, indicating sexual activity also in females at high age. Such signs were not observed in the earlier 10-year-periods 1977-1986 and 1987-1996 (Paper II).

The uteri of the two oldest females (animals no. 8 and 11) showed leiomyomas, but the uneven age distribution of the present material gives no hint of the prevalence of this tumour.

3.10.1.3. Statistics of colonic and adrenal lesions

Non-pooled data on three variables, age, colonic ulcers and adrenocortical hyperplasia, on the grey seals found dead at shore, obtained as fishery by-catch, and hunting, were used in statistical analyses. The analyses concern the post-pup stage yearlings and the ≥1-year-olds of which pooled records are presented in Tables 15 and 16. The Mann - Kendall trend test, described in Helsel & Hirsch (1995) was used. A significant positive correlation ($p<0.001$) was found between adrenocortical hyperplasia and age in both males and females. There was a significant positive correlation between colonic ulcers and age in males ($p<0.001$) but not in females. There was a significant positive correlation between colonic ulcers and adrenocortical hyperplasia in males ($p<0.001$) and females ($p<0.021$).

3.10.2. Grey seals at the Swedish West Coast

One grey seal male, aged 6 years was found dead at the northern Swedish West Coast on April 3, 2002. The carcass showed a rather severe autolysis, but certain observations were done: the animal was in a normal nutritional condition and showed no signs of drowning or signs in the lungs or lymphatic organs consistent with those occurring in Distemper. The only finding recorded was an acute colitis, the dignity of which was not judged to be the cause of death. The ileocaeco-colonic region showed no hookworms and no signs of colonic ulcers. The adrenals showed a moderate cortical hyperplasia.

3.10.3. Baltic ringed seals

The material of ringed seals was small as compared to the grey seals. Sixteen Baltic ringed seals (eight females and eight males) were investigated during 1997-2002. All of them were by caught at fishery. Age determination by tooth examination is not yet finished in this species; why age classification into yearlings, subadults (1-3-year-old) and adults (≥4-year-old) was done by estimation, as described in Material and Methods.

Of the females, one was a yearling, five were subadults and one was adult, and of the males, five were yearlings and three were subadults.

Colonic ulcers and other lesions

The occurrence of colonic ulcers is shown in Table 18. There were no animals with severe (Grade 3) lesions. Of the two adult females, one sampled October 5, 1997 showed signs in the uterus of an interrupted pregnancy. This female showed a slight adrenocortical hyperplasia. The other female, sampled May 23, 2001 showed a healthy uterus with signs of a normal pregnancy 2000-2001. This female showed a moderate adrenocortical hyperplasia and as a sub-finding, a unilateral renal atrophy.

One of the subadult males, having a Grade 2 colonic ulcer showed a slight adrenocortical hyperplasia.

Table 18. *Number of Baltic ringed seals in different age classes with no (Grade 0), slight (Grade 1) and moderate (Grade 2) colonic ulcers in females and males collected 1997-2002.*

	Females				Males			
Age, years	n, cases examined	Grade 0	Grade 1	Grade 2	n, cases examined	Grade 0	Grade 1	Grade 2
<1	1	1	0	0	5	5	0	0
1-3	5	2	3	0	3	1	1	1
≥4	2	0	2	0	0	0	0	0
S^n	8	3	5	0	8	6	1	1

3.10.4. Baltic harbour seals

As recorded in chapter 2.17.3, Table 13, age classification and age determination of the 11 harbour seals (three females and eight males), showed two female and three male yearlings, one female and two male subadults and three adult males.

Pathological changes and causes of death

Ways of collection and causes of death of these animals are summarised in Table 19.

Table 19. Harbour seals from the Baltic and Oresund, collected 1997-2002. BCF, by caught at fishery, FD, found dead on shore and FI, found ill on shore.

Sex	Case no.	Age, years	Way of collection	Cause of death
♀♀	1	<1	FD	Cause of death not confirmed
	2	<1	BCF	Drowning
	3	1	BCF	Drowning
♂♂	1	<1	BCF	Drowning
	2	<1	FD	Verminous pnemonia
	3	<1	FI	Emaciation
	4	2	BCF	Drowning
	5	2	FD	Chronic cholangitis, chronic hepatitis, emaciation
	6	10	BCF	Drowning
	7	15-20	BCF	Drowning
	8	23	FD	Cause of death not confirmed, large facial abcess

Lesions pertaining to the BSDC were restricted to adrenocortical hyperplasia of Grade 1 in male no. 7, 15-20-year-old, and of Grade 2 in male no. 6 (ten-year-old) and of Grade 3 in male no. 8 (23-year-old). Male no. 8 also showed severe periodontitis.

Of the four animals found dead at shore, one yearling (male no. 2) died from verminous pneumonia, one subadult (male no. 5) from chronic cholangio-hepatitis, while in the third case cause of death could not be confirmed. Part of the carcass of this animal, a yearling (female no. 1), had been consumed by scavengers. As regards the fourth case, the 23-year-old male only organ samples were available for examination. As judged from examination of a piece of skin and blubber, this male had probably died from emaciation, secondary to a large profound abscess in the facial region. The male yearling (no. 6) that was found ill at shore was emaciated.

As a curiosity, it can be mentioned that the last weeks of life for the 15-20-year male (no. 7) were of great interest for the media. He had moved astray, during the summer 1998, from the Baltic via Stockholm, to Lake Mälaren and unfortunately died there in a fishing-net.

Two harbour seals (female no. 2 and male no. 5) were examined during 2002. These animals showed no signs of Distemper. They were both from Öland in the southern Baltic and collected on August 7 and September 7, respectively, i.e. during the course of the epizootic.

When looking back upon the period when the author was responsible for the SWISP project (1997-2002) it should be pointed out that signs of colonic ulcers never have occurred in harbour seals, neither in those from the Baltic nor in those from the Swedish West Coast.

3.10.5. Harbour seals on the Swedish West Coast

Besides victims of the second Distemper epizootic in 2002, material from five harbour seals only, has been sent for investigation during 1997-2002. Whole-body investigation was performed on a female yearling in which other changes than emaciation (the cause of death) were not observed. Sample material investigation included normal reproductive organs from four females, which had been shot at legal hunting.

3.11. Distemper epizootic in 2002

The first harbour seals victims of Distemper in 2002 were found on May 4th. As in the distemper epizootic in 1988 the first cases appeared on the Danish island Anholt in Kattegat. The disease then spread and carcasses were found on the neighbouring Danish islands Laesø and Hesselø on May 21 and May 29 respectively and on the Swedish West Coast, at Varberg and Koster on May 28 and in the Oslo Fjord of Norway on June 22. The first cases in the Baltic were observed on July 18. Dates when animals appeared in different areas of the Wadden Sea area were; June 16 in the Netherlands, July 17 in the Lower Saxony, August 26 in Schleswig-Holstein and August 30 in the Danish area. Cases appeared at Helgoland and in France/Belgium on August 11 and 13 respectively and in Limfjord, Denmark on September 19. In the United Kingdom, cases were observed in the Wash on August 12, in the Orkneys, and North Ireland on September 17 and 21 respectively and in North and West Scotland and the Shetlands in the beginning of October. Based on reported fatal cases the 2002 epizootic lasted from the beginning of May (Denmark) to mid December (Scotland). The above data are from Härkönen, personal communication, and from Härkönen *et al.* (2004).

The report by Härkönen *et al.* (2006) concerns the epidemiology of the 2002 PD epizootic with revised data from the 1988 epizootic. A total of 17 713 and 20 462 carcasses of harbour seals were sampled in 1988 and 2002, respectively. These authors estimate, via pre- and post-epizootic censuses, that the mortality in the disease was >23 000 in 1988 and >33 000 in 2002, which represented 28% of the entire North Sea population on both occasions. There was a considerable variation of mortality rates during the 2002 epizootic ranging from 66% in the Northern Skagerrak to <1% in Scotland and the Shetlands.

To verify the spread of the epizootic in Swedish waters necropsies on harbour seals that were found dead at shore were performed at the SMNH, the Museum of Natural History in Gothenburg and the Kristineberg Marine Research Station. Further harbour seals were shot due to severe respiratory distress during two field expeditions: in the Kattegat (Bua-Varberg) on July 17-22, and in the Northern Skagerrak (Hamburgsund-Väderöarna) on August 21-27. Age was estimated to the level of age-class into yearlings, subadults (1-3-year-olds) and adults (≥4-year-olds).

Carcasses of seven harbour seals (four females and three males) were in a condition allowing investigation to confirm the spread of the epizootic. The females were one aborted foetus, one subadult and two adults. The males were one subadult and two adults. Six of the animals showed macroscopic lesions consistent with those occurring in Distemper (see Paper VI), while the seventh, the female foetus showed no signs of that kind.

The 20 harbour seals (seven females and 13 females) that were shot were necropsied soon after death. They included five female and five male subadults and two female and eight male adults all of which showed macroscopic lesions consistent with those occurring in Distemper.

3.12. Bacteriology

The bacteriological examinations revealed different species of potentially pathogenic bacteria as signs of infection. Isolation of such bacteria in connection with pathologic findings, were considered as a specific infection. Negative results, or results indicating growth only of saprophytic bacteria etc., are exceptionally commented.

The signs of specific bacterial infections were concentrated to the female reproductive organs and ulcers in the ileocaeco-colonic region in grey and ringed seals. Harbour seals have so far, as mentioned earlier, not shown similar lesions. Findings in other organs are described separately.

3.12.1 Grey seals

Female reproductive organs

Sixteen of the 17 grey seals examined were sexually mature. Ten of 16 animals showed uterine obstructions (occlusions or stenosis). Of these ten animals, one showed pyometra (see Paper I, Fig. 5). Of the other six sexually mature females, two showed less prominent endometritis, two showed post-abortion or post-pregnant endometrial changes, while in two there were no pathological changes of the uterus. The remaining female, a post pup-stage yearling, showed signs of vulvitis.

The examination revealed an infection suspected to be specific in the uterus of three cases:

Beta-haemolytic streptococci, Lancefield group C: one case with uterine occlusion

Beta-haemolytic streptococci, unspecified: one case with slight purulent endometritis

Beta-haemolytic streptococci, Lancefield group G: one case in a uterine state after normal pregnancy.

The uterus of the female, mentioned above, described in Paper I, showed no growth of bacteria.

Male reproductive organs

Samples from one male only, 12-year old, with chronic orchiepididymitis were examined. *Proteus mirabilis* in close to pure culture was isolated.

Intestine

Samples from ileocaeco-colonic regions were collected from ten females and nine males. Seventeen of these showed slight to severe colonic ulcers, five of which were fatal. Of the remaining two animals, one showed an acute colitis and one showed no intestinal lesions. Samples from the regional lymph nodes were collected from eight cases.

Bacteria belonging to the normal intestinal flora were isolated from most samples except from six animals with colonic ulcers, from which the following potent pathogens also were isolated:

Streptococcus zooepidemicus: two cases

Beta-haemolytic streptococci, Lancefield Group G: one case

Beta-haemolytic streptococci, unspecified: one case

Streptococcus sp: one case

Mycobacterium paratuberculosis: one case

Spread of bacteria to the regional lymph node was found in the two cases infected with *Streptococcus zooepidemicus*.

Other organs

Specific infections were found in 18 of the 53 animals examined. The results are recorded in Table 20.

Table 20. *Pathogenic bacteria and number of grey seals from Swedish waters infected (^{n}inf), signs of disease and number affected (^{n}aff) at bacteriological examination of some organ systems 1977-2002.*

Pathogens	^{n}inf	Sign of disease	^{n}aff
Beta-haemolytic streptococci, Lancefield group C, F and G, and non-typed	9	Tonsillitis	3
		Pharyngitis	1
		Respiratory tract inflammation	1
		Regional lymph-adenitis	1
		Bacteraemia	1
		Septicaemia	2
Archanobacterium spp.	2	Purulent broncho-pneumonia	2
Aeromonas hydrophila	2	Septicaemia	2
Escherichia coli	2	Septicaemia	1
		Abscess of mesenteric lymph node	1
Pasteurella spp.	2	Local dermatitis	1
		Tonsillitis	1
Edwardsiella tarda	1	Respiratory tract inflam-mation, bacteraemia	1

3.12.2 Ringed seals

Female reproductive organs

All the 12 ringed seals were sexually mature, seven of which showed uterine obstructions, primarily occlusions. One of the seven seals showed stenosis of one uterine horn, occlusion of the other and a severe purulent endometritis. Of the remaining five seals, one showed a purulent endometritis, in one the uterus was in a state of implantation, and three showed macroscopically normal but sterile uteri.

The examination revealed pathogens suspected to be infectious in the uterus of four cases:

Beta-haemolytic streptococci, unspecified: one case with uterine occlusion.

Beta-haemolytic streptococci, unspecified, and *Archanobacterium* spp.: one case with uterine occlusion.

Archanobacterium, unspecified: one case, mentioned above, with stenosis/ occlusion and purulent endometritis.

Pseudomonas spp.: one case that showed to be in a state of implantation.

Intestine

The two animals examined were adult females showing colonic ulcers of a slight and a severe degree respectively.

Beta-haemolytic streptococci, unspecified, were cultured from the ulcer region of the severely affected animal.

Other organs

Two of the seven animals examined showed specific infections. Both animals had subcutaneous abscesses *Staphylococcus aureus* was isolated in samples from one case and *Archanobacterium pyogenes* in samples from the other.

3.12.3 Harbour seals

Female reproductive organs

Two adult females were examined, both showing purulent vaginitis. Bacteriological examination revealed no specific infection.

Other organs

Four of the 20 animals examined showed specific infections.

Alpha-haemolytic streptococci, Lancefield group C: pneumonia (one case).

Alpha-haemolytic streptococci, Lancefield group F: pneumonia (one case).

Beta-haemolytic streptococci, non-specified: thyroidal abscess (one case).

Beta-haemolytic streptococci, non-specified: septicaemia (one case).

3.12.4 Conclusive aspects

This investigation gives some information about commonly occurring potential pathogenic bacteria involved in disease in seals from Swedish waters. Of these, beta-haemolytic streptococci dominated; followed by other streptococci and *Archanobacterium* spp. *Aeromonas hydrophila* was isolated in two cases of septicaemia in grey seals, one of which was reported by Krovacek *et al.* (1998). *Aeromonas hydrophila* was frequently isolated in different organs reflecting the aquatic environment of the seals.

3.13. Parasitology

As mentioned in Material and Methods, chapter 2.9., the material included 38 Baltic grey seals, nine Baltic ringed seals and 17 harbour seals, the latter from the Baltic, Oresund and the Swedish west coast.

3.13.1 Baltic grey seals

During the first phase of the investigation period organ samples for parasitological examination were taken when parasite infection was suspected. Since the occurrence of different parasite species was found to be rather regular from case to case the sampling later on was done more randomly.

Stomach and intestine

Stomachs from 36 grey seals (15 females and 21 males) and intestines from 32 of these animals (14 females and 18 males) were examined. The occurrence of parasites in two age classes (<1 and ≥1-year-olds) of females and males is recorded in Table 21.

Stomach: As shown in Table 21 ascarid infection was found in all 36 cases. Co-infection Ascarid-Corynosoma sp. was found in two of these cases. There was Porrocaecum sp. infection in 20 and Contracaecum sp. infection in 12 cases, in one of these cases as a co-infection. Anisakis infection was found in three cases.

Intestine: As shown in Table 21 Corynosoma sp. infection dominated and was found in 26 of the 32 cases. The finding of ova of two liver flukes sp. (Pseudamphistomum truncatum and an Opistorchis sp.) from the intestine of one animal (necropsied in 1996) is interesting and is commented further (see Discussion: Parasites, fish and seals).

Table 21. *Presence of parasite species in stomachs of two age classes of Baltic grey seal females and males.*

Age, years	Stomach		Intestine	
<1	Porrocaecum sp.	4	Corynosoma sp.	3
	Contracaecum sp.	2	Corynosoma sp./Contracaecum sp.	1
			Parasites not found	1
≥1	Porrocaecum sp.	14	Corynosoma sp.	14
	Contracaecum sp.	9	Corynosoma sp./Ova of ascaroidea	1
	Porrocaecum sp./Contracaecum sp.	1	Corynosoma sp./Porrocaecum sp.	5
	Porrocaecum sp./Corynosoma sp.	1	Corynosoma sp./Contracaecum sp.	1
	Contracaecum sp./Corynosoma sp.	1	Porrocaecum sp.1	1
	Anisakis sp.	3	Contracaecum sp.	2
	Ascarid, not specified	1	Corynosoma sp./Ova of two liver fluke sp: Pseudamphistomum truncatum and an Opistorchis sp.	1
			Parasites not present	2
S^n		36		32

Other organs
Samples of other organs from 38 animals (16 females and 22 males) were examined. No viable parasites were found in samples of the liver (nine cases) or in the lungs (22 cases). Larvae of Trichinella sp. were not found in the diaphragm of (20 cases). Mite infection of the rhinal mucous membrane by Halarachnidae was confirmed in samples from three animals: one female 33 years old and two males, seven and eight years old.

3.13.2 Baltic ringed seals

Organ samples from nine Baltic ringed seals were examined.

Stomach
Stomachs of seven of the nine animals were examined: one female and two male yearlings and four female aged ≥1 years. Parasite species were found in three animals while in three animals parasites were not found (Table 22).

Intestine
Intestines of all nine animals were examined: the same yearlings as the abovementioned, and five female and one male aged ≥1 year (Table 22).

Table 22. Presence of parasite species in stomach and intestine of two age classes of Baltic ringed seal females and males collected during 1977-2002.Table 21.

Age, years		Stomach	Intestine
<1	Porrocaecum sp. and Corynosoma sp.	1	1
	Parasites not found	2	
	Corynosoma sp.		2
≥1	Porrocaecum sp.	2	
	Parasites not found	2	
	Corynosoma sp.		3
	Corynosoma sp. and a Cestode: Diphyllobothridae sp.		1
	Plerocercoids of a Cestode: Schistocephalus solides		1
	Corynosoma sp. and a Cestode, unspecified		1
S^n		7	9

Other organs
Heart blood: Lung worms-metastrongylidae (Pseudalis sp.) were found in a female yearling. Nematodes (Halocerus sp.) were found in a male yearling and lung worms-metastrongylidae (Tornyros sp.) in a ≥1 year-old male.

No viable parasites were found in other organs: In total samples from nine animals were examined including lung from eight, liver from two, and diaphragm from six. Samples of liver from two, diaphragm from six and lung from eight of the nine animals were examined. No viable parasites were found in these samples.

3.13.3 Harbour seals from the Baltic, Oresund and Swedish west coast

The material included organ samples from altogether 17 animals which were subjected to examinations of different organs as specified below:
Stomachs of 11 animals were examined: from two female and one male yearling and four females and four males aged ≥1 year (Table 23).
Intestines of eight animals were examined: one of the female yearlings, four females and three males aged ≥1 year (Table 23).

Table 23. Presence of parasite species in stomachs and intestines of two age classes of Harbour seal females and males from the Baltic, Oresund and the Swedish west coast collected during 1977-2002.

Age, years		Stomach	Intestine
<1	Anisakis sp.	1	
	Anisakinae/Porrocaecum sp.	1	
	Porrocaecum sp.	1	
	Parasites not found		1
≥1	Porrocaecum sp.	3	
	Porrocaecum sp. and Contracaecum sp.	2	
	Anisakis sp.	1	
	Anisakinae sp.	1	
	Corynosoma sp.	1	3
	Corynosoma sp. and Anisakinae sp.		1
	Corynosoma semerme and larvae of Anisakis marina		1
	Corynosoma sp. and a trematode: Rossicotrema sp.?		1
	Acanthocephala sp.		1
S^n		11	8

Other organs
Samples of liver from one, diaphragm from three and lung from nine of the 17 animals were examined. No viable parasites were found in these samples.

3.14. Virology

During the 1988 PD epizootic PDV was not isolated as regards the Swedish material and neither were there signs of infection with influenza virus. However, during that year (1988) 32 out of 49 seals investigated from Swedish waters was shown to be positive for PDV by the use of immunofluorescens (B. Klingeborn, personal communication).

Later with increasing awareness of distemper infection and with improved diagnostic methods Distemper virus was isolated in tissues from most harbour seals with morphologic signs of PD during the 2002 epizootic using PCR technique.

The interference of antibodies raised against PDV and the lack of fresh tissue samples are possible reasons why PDV or virus RNA was not detected in spite typical pathological signs of PDV- infection (Müller *et al.*, 2004).

4. Discussion

The Baltic Seal Disease Complex (BSDC) consists of lesions in several different organs belonging to widely different organ systems such as skin and bones, intestines and kidneys. This raises two main questions.

Is there a single common aetiology or are there several concurrent factors behind the disease complex? How are the different changes of organ function correlated to each other? Are they all independent or are there one or more primary changes which give rise to or influence the others?

Organ lesions

Adrenals

One of the most prominent changes in the BSDC was adrenocortical hyperplasia. It was present in all animals above 15 years of age (II: table 9:5-9) and was statistically related to inter al. the renal lesions described in Paper V. It was suggested as a crucial change, explaining other organ lesions (I).

Macroscopic examination of the glands has shown an evenly distributed thickening of the adrenal cortex or a nodular hyperplasia but no signs of primary adrenal tumours. Microscopically the proliferated cells showed to be located within the Zona fasciculata and reticularis layers of the adrenals.

Do the proliferative adrenal changes observed in the Baltic seals reflect an increased activity by adrenal hormones?

Such effect, Hyperadrenocorticism, is characterised by an elevated blood pressure and changes in plasma glucose levels, sodium and potassium levels in the blood, increased blood lipid and cholesterol concentrations and activities of sex hormones. Biochemical analyses of these parameters have not been made. Single animals only were in a fresh state of preservation making such analyses meaningful. This discussion must therefore rely exclusively on morphological studies.

Three types of adrenocortical hyperfunction have been described in the literature:

a. Hyperaldosteronism
b. Hyperandrogenism (Adrenogenital syndrome)
c. Hypercortisolism (Cushing's syndrome)

Increased aldosterone activity causes sodium retention and increased potassium excretion. Characteristic lesions are observed in the renal epithelial cells with vacuolisation of the cytoplasm (Trump & Bulger, 1968.). Changes of this type were not observed in the Baltic seals. Tubular cell proliferations as described in the BSDC (Paper I and V) have, to the author's knowledge, not been reported in hyperaldosteronism.

There were no signs of an increased androgen activity in the reproductive or any other organs of the seals.

Which is the cause of the adrenocortical hyperplasia?

Signs of adenomatous proliferations in the hypophysis have not been observed at macro- and microscopic examinations of Baltic seals - light microscopy has been performed on hypophyses from 37 animals, some of them rather well preserved and having huge adrenocortical thickening. It therefore seems improbable that the adrenocortical hyperplasia, which is observed in the seals, is due to a hyperstimulation from the hypophysis.

Signs of effects on the hypophysis exerted by a possible adrenal hyperfunction are however also of great interest. Collaboration between the author and the Dept. of Pathology at the Academic Hospital in Uppsala is in progress and is focused on tests of antibodies against hypophyseal hormones.

Findings at experimental research on PCB exposure of the hypophysis is described in the following under Female reproductive organs.

Direct influence of environmental pollutants ?

The lipid soluble organochlorines interfere with the steroid metabolism. Elevated glucocorticoid levels and ultrastructural signs of hyperactivity of the adrenal Zona fasciculata was found in rats fed PCB (Wasserman *et al.* 1979, Wasserman & Wasserman 1972. Copeland and Cramner (1974) found adrenal enlargement but no effects of steroid production in dogs exposed to DDT. Adrenal enlargement was also observed in mink after exposure of relatively large concentrations of planar PCBs (Aulerich *et al.* 1985, 1987). Other authors, on the other hand, have reported no hyperplasia and inhibition of adrenal corticosteroid production in rats treated with low levels of toxaphene, DDT and technical PCB (Aroclor 1254) during 35-155 days (Young *et al.* 1973, Mohammed *et al.* 1985, Byrne et a. 1988). The mode of action of organochlorines such as DDT and PCBs on the adrenal gland is unclear (Gregory and S:t Cyr 2003).

The elevated gluccocorticoid levels seen in rats fed PCB by Wasserman *et al.* (1979) was believed by these authors to explain at least partly a reduced activity of the immune system. DDT is also known to lower the immune response, although to a minor degree (Wasserman *et al.* 1979,Wasserman *et al.* 1973 b).

The role of an eventual defect immune response and the inflammatory processes above all in the intestines will be discussed in the following.

A stress reaction?

It is well known that prolonged stress, such as severe chronic illness may induce an enlargement of the adrenals in humans to more than twice their normal weight (Cotran *et al.* 1999a). The severe ulcerative lesions in the intestines, the skull bone lesions with loss of teeth with impaired both ingestion and digestion of food and the final emaciation with loss of insulating blubber are indication that animals with advanced changes had suffered from a severe stress reaction. The result from the compiled findings at necropsy during 1997-2002 is a further support of this cause. This study showed a statistically significant connection between colonic ulcers and adrenocortical hyperplasia in Baltic grey seals (Ch. 3.10.1.3).

There is no solid evidence that a direct influence by pollutants in the Baltic biota is the cause of the adrenocortical hyperplasia in the seals. Multiple chronic organ lesions indicate that the animals suffered from a severe stress reaction, which is the most probable cause of the adrenal change.

Female reproductive organs

The continuous observations during the passed four decades indicate that the main cause of the decline in the Baltic seal population was a severely reduced reproductive capacity.

Uterine occlusions and stenoses, which are prominent features of BSDC, may be associated with pregnancy (Helle *et al.* 1976b, Helle 1980, Bergman & Olsson, 1985). Early in this investigation the uterus of three grey seals showed retained foetal membranes, with presence of necrotic foetuses in two of them (Papers I and II), as indications of abortion and foetal death. Interrupted pregnancies are common after experimental PCB studies in mink *(Mustela vison)*, see e.g. Aulerich and Ringer, 1977; Jensen *et al.* 1977; and Kihlström *et al.* 1992. It has also been shown that exposure to PCB and related compounds can induce abortion in monkeys (Altman *et. al*. 1979; McNulty, 1985).

During the last 15 years, knowledge has increased about effects by PCBs and related compounds on the hypophysis, based on studies on experimental animals (rodents, fish and birds) and cultured pituitary cell lines. Such compounds have been shown to alter the regulation of hormones of the hypothalamic-pituitary-thyroid axis, the hypothalamic-pituitary-gonadal axis and the hypothalamic-pituitary-adrenal axis. Desaulniers *et al.,* 1999, reported about effects in rats on anterior pituitary and thyroid hormones, induced by the non-*ortho* substituted coplanar chloro-biphenyl 126 (CB-126), regarded as the PCB congener having the highest TCDD-TEF value (Krishnan and Safe, 1993). The di-*ortho* substituted CB-153 has a very low TCDD-TEF value but nevertheless it has been shown to give oestrogenic effects (Li *et al.* 1994). The report by Desaulniers *et al.* 1999 includes a comprehensive summary of the literature regarding PCBs and the pituitary. In a recent report, the effects of perinatal exposure to environmentally relevant doses of CB-153 and CB-126 in female goats were studied (Lyche *et al.* 2004). CB-153 was found to affect the reproductive function in the females whereas the coplanar CB-126 did not produce any effect (Lyche *et al.* 2004).

Studies on Baltic grey seals have shown high concentrations of CB congeners with 2-4 chlorines in ortho-position to the biphenyl bond in the past whereas concentrations of both coplanar PCBs as well as dioxins were low or even not at detectable concentrations (Haraguchi *et al.* 1992, Bergek *et al.* 1992). On basis of these results it was suggested that PCB molecules with 2-4 chlorines in *ortho*-position were responsible for the lesions and poor reproduction of Baltic grey seals and not the coplanar PCBs and dioxins (Olsson *et al.* 1992). The more recent results by Lyche *et al.* seem to support this suggestion and it may be so that oestrogenic effects caused by non coplanar PCBs are of importance to understand the earlier much reduced reproductive capacity in the Baltic seals.

A high prevalence of uterine leiomyomas was found in old Baltic grey seals (Paper I and II). It has not been possible to obtain an age-matched material from animals not exposed to similar high levels of contaminants as in the Baltic and there are no investigations about the occurrence of such tomours in seals inhabiting other areas. Leiomyoma is common among dogs (Kennedy and Miller, 1993) but is uncommon in other animals. It can not be excluded that the high prevalence of leiomyomas is a common feature in old animals unrelated to environmental pollutants. Increased oestrogen activity has been considered as a main promoter of leiomyoma growth (Crum, 1999).

A recent experimental investigation using Baltic grey seal's myometrial cells supports the hypothesis that certain organochlorines can influence growth of uterine leiomyomas in this species (Bäcklin *et al.* 2003). The possibility that leiomyoma growth in elderly grey seals may be an effect of exposure to such substances must therefore be considered.

Integument

Chloracne-like changes similar to those present in one grey seal female (Paper I): thin epidermis, hyperkeratosis, cystic dilation of hair follicles, and atrophy of sebaceous glands, have been described in connection with adrenal hyperfunction and other forms of endocrinopathy (Muller *et al.* 1983).

Experimental investigations of the effects of PCBs and DDTs on animals have also shown effects on integuments. Abnormalities of nail/claws have been observed in European ferrets *(Mustela putorius furo)* (Bleavins *et al.* 1987) and American mink *(Mustela vison)* (Aulerich *et al.* 1987) which were fed PCBs.

A comparison with the Rice oil disease, or Yusho, in Japan in 1967 may also be made. People who ingested rice oil, which was accidentally contaminated with PCB showed almost consistent skin lesions and affection of nails, so called chloracne of the same character as those found in the grey seals, mentioned above, and also pigment anomalies (Kikuchi & Masuda, 1976; Tryphonas *et al.*, 1984). Similar skin lesions have also been observed in monkeys after oral exposure to PCBs or chlorinated dibenzodioxine (Barsotti and Allen, 1975, Allen *et al.* 1977). The lesions of the claws and claw regions were less serious than those seen in the seals. These changes, above all the deformation of the claws, must have involved severe problems with the nutrition of the animals, which could have had some pertinence in the development of the BSDC.

Bone

The cause of the severe lesions of skull bones in grey seals (Paper III) is, judging by the macroscopic appearance an inflammatory process similar to the parodontitis seen in other animals and man. This may be associated with a defect immune response. The probable explanation for the utmost severe destruction of masticatory bones that is observed in grey seals (see Fig. 2, Paper III) is a primary osteoporosis, causing loss of bony support for the teeth and further aggravation of the lesion.

Recent observations on Baltic grey seals indicate an improvement and lowered prevalence of skull bone lesions since 1980. In this study (Bergman *et al.* unpublished data) grey seals born 1979 and earlier and aged 6 to 15 years showed a high frequency of skull bone lesions (28 affected out of 72: 29%). Seventeen of the 28 skull bones affected showed severe lesions. In the same age group of animals, but born 1980 and later, four out of 39 skull bones (10%) showed lesions, none of which of a severe degree.

The results on bone mineral density (paper IV) are partly in accordance with the previous results from the macroscopic examination of skull bones from Baltic grey seals (Paper III). The trabecular bone mineral density was higher in radius bones of grey seals collected 1986-1997 than in those collected 1965-1985. This finding is in accordance with the observation mentioned above (Bergman *et al.* unpublished data) showing a lower prevalence of skull bone lesions in recent years. However, a continuous decline in the cortical bone mineral density in mandibles was observed during the periods 1965-1985 and 1986-1997 compared with the findings in 1850-1955 a period of low contamination with organochlorines in the Baltic biota.

The reason for this difference in trabecular and cortical bone density is not known. It may reflect the complex and considerable change of composition and levels of contaminants in the Baltic during the last 3-4 decades. The concentrations of DDT and PCB in biota of the Baltic started to decrease during the first half of the 1970s and reached fairly low levels during the 1990s (Olsson and Reutergård, 1986; Bignert *et al.* 1999). The concentrations of later introduced contaminants, such as brominated flame-retardants, have increased and did not start to decrease until the beginning of the 1990s.

Bone changes similar to those present in Baltic grey seals have also been observed in Beluga whales from the Gulf of S: t Lawrence in Canada, where the animals are exposed to a variety of xenobiotics inter al. organochlorines (Béland *et al.* 1993).
Experimentally impact on bone by PCB (Arochlor 1254) administration has been found in rats (Andrews, 1989) who also reported changes of calcium metabolism and nephrotoxicity. There is also experimental evidence of an influence of organochlorines on skull bone periodontal epithelial cells. Render *et al.* (2000 and 2001) have shown proliferation of these cells in mink fed CB-126 or 2,3,7,8-tetra-chlorodibenzo-*p*-dioxin.

Osteoporosis is one of the cardinal features in states with long term elevation of of glucocorticosteroid levels in humans (Sommers, 1977) and domestic animals (Feldman, 1983). Disturbed interrelation between the metabolism of calcium, phosphorus, vitamin D, parathyroid hormone and calcitonine are other well known causes of bone demineralisation (Palmer, 1993, Rosenberg, 1999). Experimentally reduced levels of active vitamin D metabolites have been found in rat dams and offspring exposed to a PCB mixture (Lilienthal *et al.* 2000). Subnormal levels of these substances may be a cause of the demineralisation in the Baltic grey seals.
The mechanism behind the bone lesions in BSDC may be complex and is not sufficiently known at present. The importance of endocrine factors in the pathogenesis of the bone lesions in the Baltic grey seals is therefore uncertain.

Kidneys

Glomerular lesions
A detailed discussion of the similarity between the specific glomerular changes in BSDC: glomerular capillary wall nodules (CWN) and glomerular capillary wall thickening (CWT), and different lesions in human renal diseases is presented in Paper V. The closest similarity is between the renal changes of BSDC and some types of human diabetic glomerulosclerosis. We have not been able to study the glucose metabolism in the seals since fresh blood was available only in a few cases. There were no other renal vascular changes in the BSDC, such as arteriolar hyalinisation, indicating a disturbed glucose metabolism. Presence of hyaline nodules in the glomeruli is a prominent feature of human diabetes mellitus with exudative lesions but these lesions are not specific for this disease (Churg *et al.* 1995).

There were no immune deposits in the glomerular capillary walls or in the mesangial cells, which could be demonstrated at electron microscopy.

The effects of oestrogen on the kidneys have been the subject of several experimental investigations (see Paper V). The results are inconclusive. Renal changes have been observed but they are variable and seem to be species-specific. They are not of the same kind as those of BSDC. There are no indications that the glomerular changes in BSDC are dependent on oestrogen hyperactivity.

The question of the pathogenesis of the glomerular lesions must be left open.

Tubular lesions
The tubular cell proliferations (TCP) were the most conspicuous of the renal changes. Metaplastic changes in the renal tubular epithelium have been described in dogs in connection with feminisation due to Sertoli cell tumours in the testes (Lindberg *et al.* 1976) and in connection with an ovarian granulosa cell tumour (Wouda et al, 1978). Similar changes have also been found in dogs after long-term administration of synthetic and natural oestrogens (Zayed *et al.* 1998). The epithelial cells were consistent with squamous epithelium in these reports, however, and are not equivalent with the changes in the Baltic seals. Bruckner *et*

al. (1974) after PCB (Aroclor 1242) administration to rats described hyperplasia of epithelial cells of the kidney, however it was restricted to the papillary epithelium. The electron microscopic investigation showed the cells to be of a type present in the normal epithelium of the collecting ducts called intercalated cells (Trump and Bulger, 1968). They have a high activity of H+/K+-ATP-ase and are engaged in the ion transport across the tubular wall (Giebish and Wang, 1996). We have not been able to investigate this transport in the seals and have therefore no possibility to ascertain if the cell proliferations are endocrinologically active.

Except for the descriptions made in paper I and V renal tubular proliferations of this appearance have, to our knowledge, not been reported previously in animals or man. It is tempting to try to find a key to the aetiology of these lesions and to put them in connection with the other features of the BSDC. There was a positive correlation between grades of the three main types of renal changes (CWT, CWN and TCP) except for CWN and TCP in males (Paper V: Table 4). They were age related, except for CWN in males (Paper V: Table 2). In Paper V: Table 3 the interrelations between grades of the three renal disease variables and grades of three variables in other organs (adrenal weight (ADW), colonic ulcers (COLU) and aortic wall changes (AWC) were also calculated. Adrenal weight correlated well with the renal lesions except for CWN in males. Colonic ulcers were well correlated with the renal lesions except for CWT in males.

Sudanophilic substance is normally present in the tubular epithelium, regularly and in great amounts in cats, occasionally in pigs and rarely in horses. In other animals the presence of lipids is in the tubular epithelium, above all in larger amounts, a sign of toxic lesions. There were no lipids in the tubular epithelium of young as well as in many old animals. It is therefore improbable that the fatty changes had anything to do with age.

Vacuolisation of renal tubular epithelial cells is a common feature in hyperaldosteronism (Churg *et al.* 1995). This was not observed in our study (Paper V) and the epithelial cell proliferations present in the seal kidneys have not been observed in hyperaldosteronism.

The changes in renal arteries were similar to arteriosclerosis in other animals and man and were correlated with age. Similar changes were also found in aged Arctic ringed seals. Interstitial fibrosis and/or infiltration of inflammatory cells in the interstitium were common in old animals. It cannot be decided if the latter changes were due to old age and circulatory disturbances alone or if external aetiological factors were also involved since no age-matched controls were available.

The correlation of the specific renal changes (TCP,CWN and CWT) with age is in agreement with the assumption that they could be associated with a long time exposure to environmental pollutants. The interrelation between renal lesions and lesions in other organs in the BSDC is consistent with the presumption of a common aetiology. There are no available data that suggest that the renal changes in the Baltic seals are sequelae to an adrenocortical hyperfunction.

Vascular lesions

The vascular changes were localised in the main arteries. They were age related and similar to age related vascular lesions present in other animals and man. They seem to be independent of the BSDC. This investigation has not produced any evidence of a causal link with the disease complex.

Aetiology and Pathogenesis

Is there a single common aetiology or are there several concurrent factors behind the disease complex? How are the different changes of organ function correlated to each other? Are they all independent or are there one or more primary changes which give rise to or influence each other?

Aetiology

Impact by environmental pollutants - PCB and DDT

A serious contamination of the Baltic by DDT and PCB was first observed in the 1960s and reported by Jensen *et al.* (1969). The Swedish Museum of Natural History (SMNH) has studied temporal variations of contaminant concentrations in the Baltic since 1967 through annual collection and analysis of defined biological matrices (Olsson and Reutergård, 1986, Bignert *et al.* 1998a). The program was extended at the end of the 1970s to include the Swedish West Coast. The main matrices have been herring *(Clupea harengus)* and cod *(Gadus morhua)*, which are the main parts of the seal's staple food. The concentrations in guillemot (Uria aalge) egg have also been analysed. The guillemot egg is an important matrix with many advantages e.g., the guillemot is non-migratory in the Baltic and preys on pelagic fish (Bignert *et al.* 1995).

Through the period 1967-1995 the PCB concentrations were three times and DDT concentrations three to five times higher in the biota of the Baltic than in the Swedish West Coast waters (Bignert *et al.* 1998). The concentrations of PCB and DDT are generally lower in the Gulf of Bothnia than in the Baltic proper (Bignert *et al.* 1998a).

Concentrations of all organochlorines investigated in the matrices mentioned above have decreased since the 1970s (Olsson and Reutergård, 1986, Bignert *et al.* 1998a), DDT since the beginning of that decade, PCBs somewhat later but with a more rapid decline.

Figures 15-20 summarise the trends from late 1960s to and including 2003 of concentrations of sPCB, sDDT and tetrachloro-dibenzodioxin (TCDD)-equivalents in herring muscle or guillemot egg from six sampling locations: Harufjärden in the northern Bothnian Bay, Ängskärsklubb in the southern Bothnian Sea, Landsort in the northern Baltic Proper, Stora Karlsö, close to Gotland in the mid Baltic, Utlängan in the southern Baltic and Fladen in Kattegat, Swedish west coast. Figs. 15-20 and the map showing the locations (Fig. 21) are performed by Anders Bignert, SMNH.

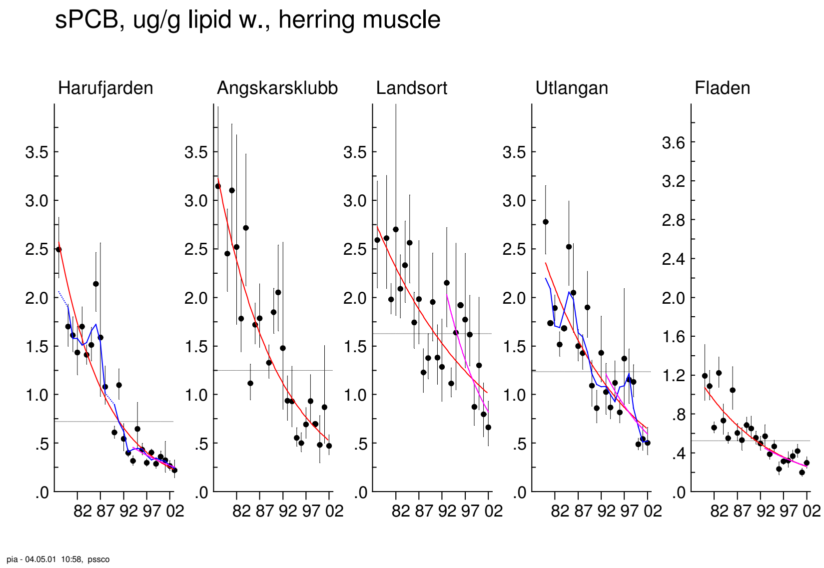


Fig. 15. Temporal trends of sPCB (ug/g, lipids) in herring muscle along the Swedish coasts, 1978(1980) –2002 (Bignert *et al.,* 2004). All time-series show significant decreasing trends. The estimated concentrations for 2003 show higher levels (about 2-3 times) in the Baltic Proper (Landsort, Utlängan) and the southern Bothnian Sea (Ängskärsklubb) compared to the Bothnian Bay (Harufjärden) and the Swedish west coast (Fladen, the Kattegatt).

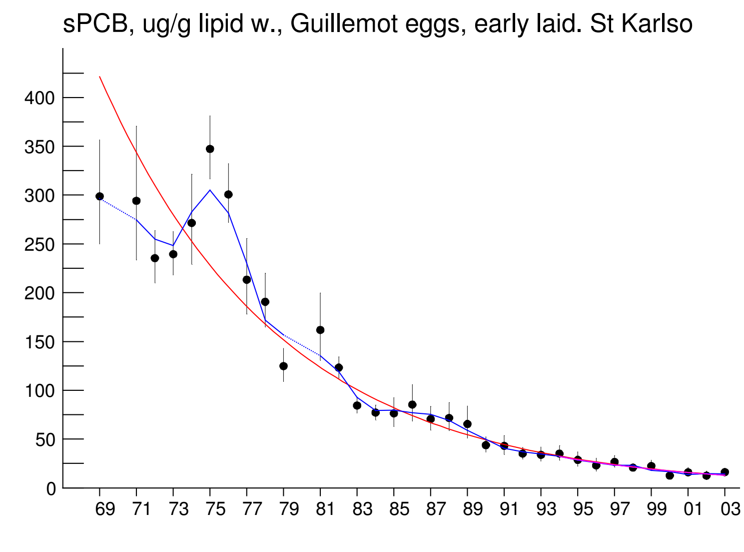


Fig. 16. Temporal trends of sPCB (ug/g, lipids) in early laid guillemot egg from St Karlsö, in the central Baltic Proper, 1969 –2003 (Bignert *et al.,* 2004).

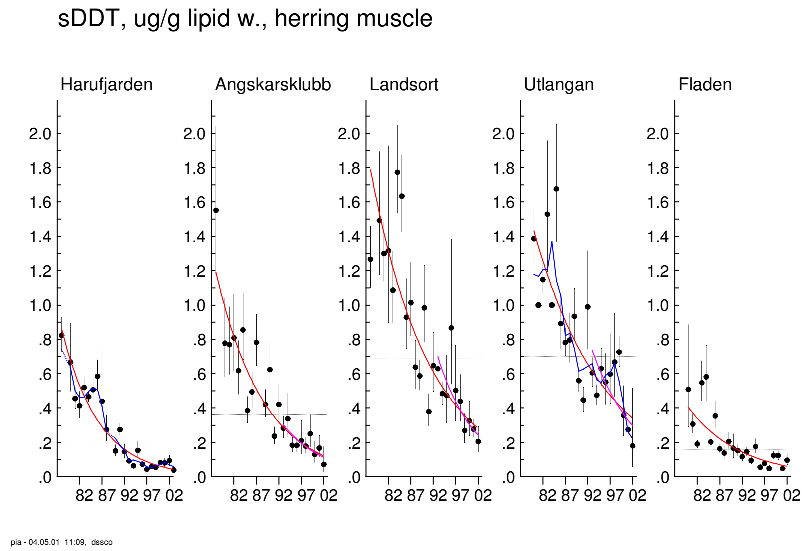


Fig. 17. Temporal trends of sDDT (ug/g, lipids) in herring muscle along the Swedish coasts, 1978(1980) –2002 (Bignert *et al.,* 2004). All time-series show significant decreasing trends. The estimated concentrations for 2003 show higher levels (about 2-3 times) in the Baltic Proper (Landsort, Utlängan) and the southern Bothnian Sea (Ängskärsklubb) compared to the Bothnian Bay (Harufjärden) and the Swedish west coast (Fladen, the Kattegatt).

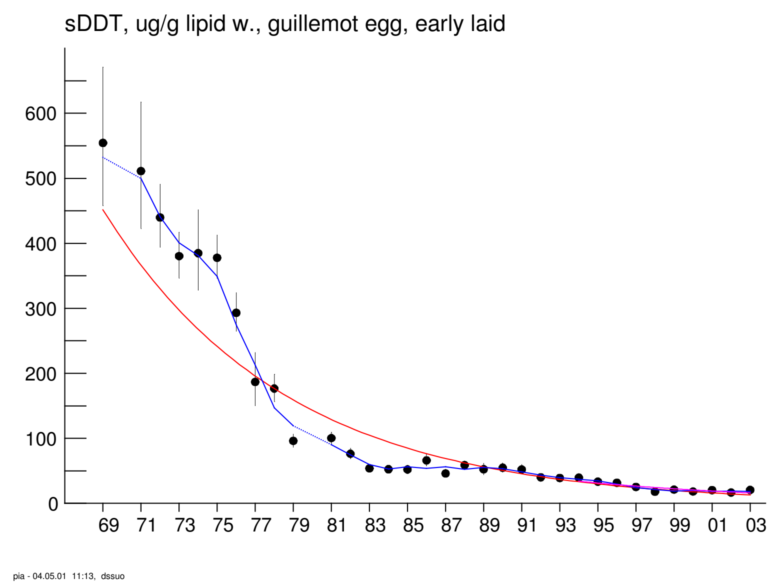


Fig. 18. Temporal trends of sDDT (ug/g, lipids) in early laid guillemot egg from St Karlsö, in the central Baltic Proper, 1969 –2003 (Bignert *et al.,* 2004).

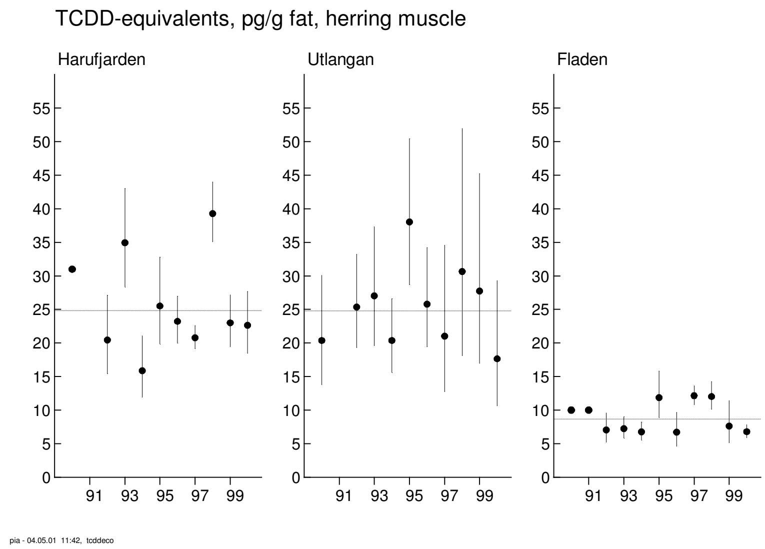


Fig. 19. Temporal trends of TCDD-equivalents (pg/g lipid) in herring muscle along the Swedish coasts, 1990–2000 (Bignert *et al.,* 2004). The time-series show no significant temporal trends. The estimated geometric mean concentrations show higher levels (about 2-3 times) in the southern Baltic (Utlängan) and in the Bothnian Bay (Harufjärden) compared to the Swedish west coast (Fladen, the Kattegatt).

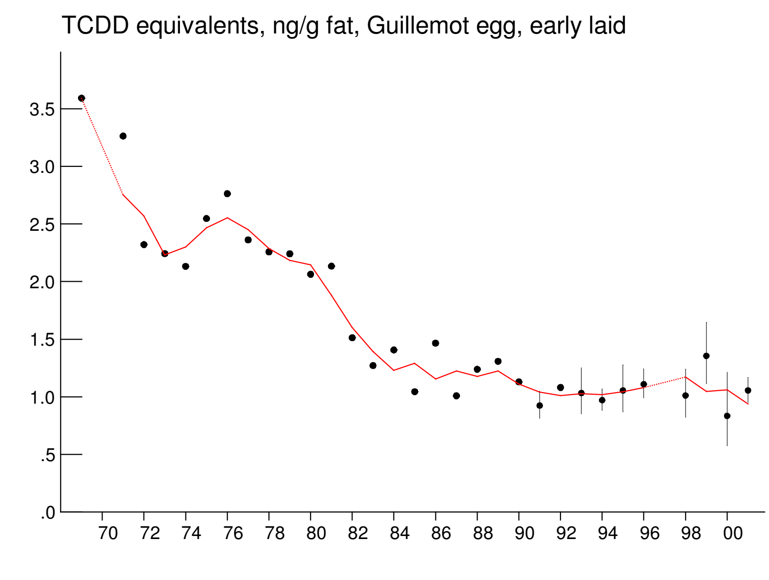


Fig. 20. Temporal trends of TCDD-equivalents (ng/g, lipids) in early laid guillemot egg from St Karlsö, in the central Baltic Proper, 1969 –2001 (Bignert et al. 2004). Initially the concentrations decrease. From about 1985 this decrease levelled out.

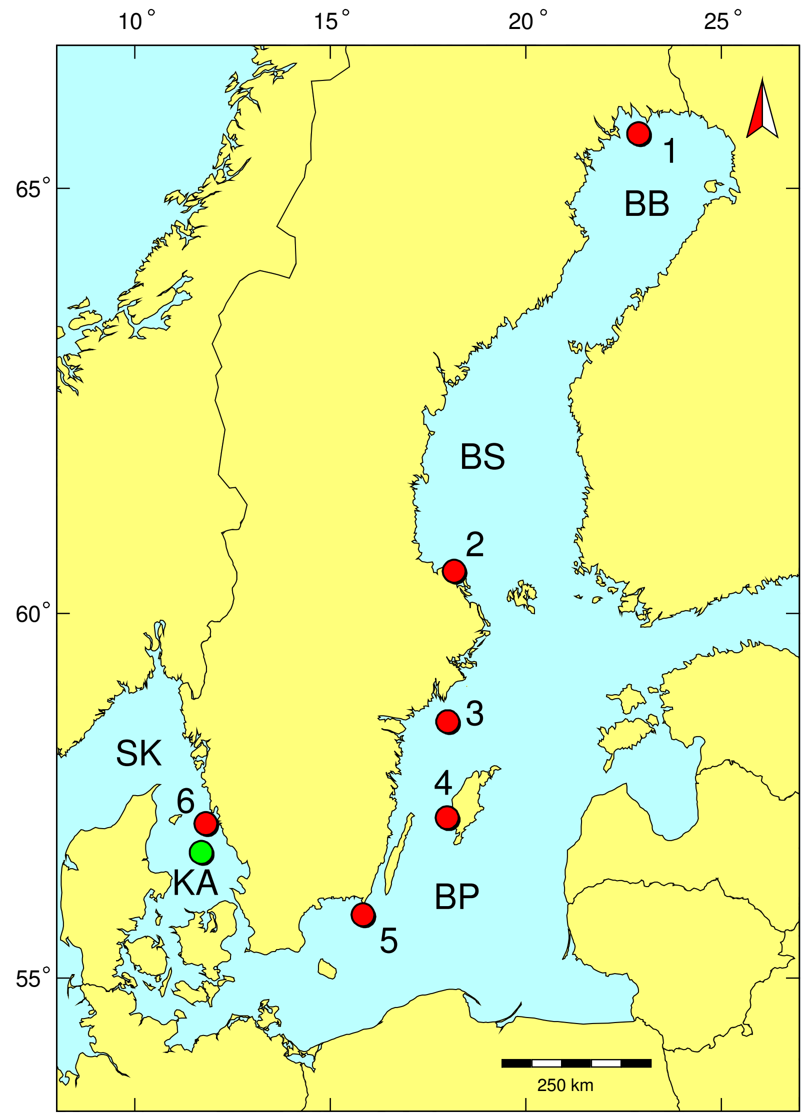


Fig. 21. Map on Swedish coastal waters. BB, Bothnian bay; BS, Bothnian sea; BP, Baltic proper; KA, Kattegat; SK, Skagerrak. Sampling locations actual in Fig. 15-20 are red-marked: 1) Harufjärden, 2) Ängskärsklubb, 3) Landsort, 4) Stora Karlsö, 5) Utlängan, 6)Fladen. The green-mark indicates the Danish island Anholt.

PCB and DDT in seals in Swedish waters

Yearlings of sub-populations of the three species inhabiting the Swedish waters were used to study the spatial distributions of contaminants. Studies of juvenile harbour seals from the late 1980s indicate that the concentrations of PCBs in specimens from the Baltic were about twice as high as in the same species from the Swedish West Coast (Blomkvist *et al.* 1992). Grey seals had the highest concentrations among the three seal species that inhabit the Baltic and Baltic grey seal females with pathological changes had a higher concentration than females without such changes (Blomkvist *et al.* 1992).

Organochlorines are lipid soluble and accumulate above all in the blubber. The metabolism and excretion of these compounds are slow. In individuals with normal nutritional condition the blubber fat concentration of such a contaminant may therefore be regarded as an index of the total body burden. The following should however be kept in mind when blubber fat concentrations of contaminants are compared with frequency and severity of different organ lesions. In emaciated animals the amount of blubber fat is much reduced, but since a lipophilic contaminant is excreted slowly its concentration in this tissue may be very high, although the total body burden may be equal with that of an animal in a normal nutritional state with lower blubber fat concentration.

In a Baltic material collected during 1969-1997 Roos *et al.* (1998) studied the concentrations of DDT and PCB in annual samples from the abovementioned biota matrices (herring, cod and guillemot egg) and from yearlings of grey seal. The concentrations of DDT were found to decrease at a similar rate in the grey seals as in the other matrices, but those of PCB remained more constant (Roos *et al.* 1998). The authors interpreted the results in the following way: a relatively high concentration of a lipophilic persistent compound observed in a grey seal yearling is largely a result of suckling, implying that its mothers had a high body burden of the compound. Consequently, the decreasing concentrations of DDT but more constant concentrations of PCB in Baltic grey seals over the period 1960s-1990s, favour the concept that PCBs, in particular, were associated with the generally low reproductive outcome in the past. This interpretation agrees with experimental results obtained at earlier studies on mink, which showed that PCB but not DDT caused reproductive failure (Kihlström *et al.* 1976, Aulerich & Ringer, 1977).

The accumulation of DDT and PCBs in prey (fish) and predator (seal) and experimental experiences indicate that intoxication with these organochlorines is an important, but not necessarily the only, factor in the aetiology of BSDC. The decreased concentration of these contaminants in the biota, which, at least partly, is paralleled by an improved health and increased seal population sizes during the last decade, supports this assumption.

Other halogenated compounds

As mentioned above the concentrations of DDT and PCB are lower in the Gulf of Bothnia than in the Baltic proper. Young ringed seals from the Gulf of Bothnia still develop uterine occlusions although at decreasing prevalence, 70% in the 1970s (Helle, 1980,a,b) and 30% in recent years (Westerling and Stenman 1992, Mattson and Helle 1995, Helle and Nyman 2000). Some data indicate that the Bothnian Sea may be more contaminated by dioxin than the Baltic proper (Bignert *et al.*, 2007a). Dioxin concentrations in guillemot eggs from the Baltic proper showed a marked decrease from the late 1960s up to 1985 when this decrease levelled out. Unlike, e.g. PCB's, the dioxin concentrations in guillemot eggs from the middle of the 1980s and in herring from the beginning of the 1990s, show no decreasing trend (Bignert *et al.*, 2007b). Dioxins are found in both ringed seals and grey seals (Bignert *et al.*, 1989, Bergek *et al.*, 1992). It cannot be excluded that the still high prevalence of occlusions in ringed seals is associated with dioxin pollution, but other chemical factors e.g. the increased environmental contamination by polybrominated compounds (Sellström *et al.* 1993) should also be considered. Another explanation would be of species-specific nature implying that the reproduction in ringed seals is more sensitive to PCB and DDT compounds than grey seals.

Heavy metals

Intoxication with cadmium (Cd), lead (Pb) or mercury (Hg) are well known causes of lesions in the liver and kidneys in man and animals. The levels of these metals in pelagic fish in the Baltic tend to be low (Johnels *et al.* 1967). In mink to which PCB was experimentally fed, higher levels of Cd and Hg were found compared with controls (Olsson *et al.* 1979). The concentrations of Cd in Baltic ringed seals did not differ from those found in Arctic ringed seals (Helle, 1981, Wageman, 1981, Dietz, 1981). Frank *et al.*, 1992 found no difference in the concentrations of Cd or Pb in the liver and kidneys of seals from various parts of the Swedish waters including the West Coast. The levels were comparable with those of seals from other areas of the world (Helle, 1981). Mercury concentrations with a maximum of 730 mg kg-1 were found in the liver of one animal in a group of aged Baltic grey seal females probably due to an age dependent accumulation both of this metal and selenium (Se) (Frank et al, 1992). The concentration of selenium paralleled that of mercury. Selenium is known to protect animals from the toxic effect of Hg (Parízek *et al.* 1971).

The histological examinations of the seal kidneys in the present material have not shown any changes compatible with mercury or other metal intoxication.
There are no indications that intoxication with heavy metals is an important factor in the aetiology of BSDC.

Pathogenesis

<u>Immunosuppression a probable factor for development of intestinal ulcers?</u>

Background of pollutants

Severe chronic inflammatory reactions are important features of the BSDC, inter al. the severe changes in the intestines and integument. There is no indication of an increased exposure to agents that provoke inflammation. It has therefore been suggested that a defect immune system is one important factor in the pathogenesis of the BSDC (Paper II: Bergman, 1999). The high prevalence of intestinal ulcers (Paper I and II) might indicate states of immunosuppression (Bergman, 1999). In Dutch experiments, when groups of captive harbour seals (*Phoca vitulina*) were fed Baltic or Atlantic herring, suppression of various cellular and antibody responses of the immune system was observed in the seals fed the Baltic herring, but not in those fed the Atlantic Herring (De Swart *et al.* 1994, 1995; Ross *et al.* 1995, 1996). These findings indicate that the food available for Baltic seals is still sufficiently contaminated to affect health. That this has a background of toxic food contamination is probable. The PCB levels in Baltic grey seals may still be high enough to influence negatively their immune system including, e.g., the sequence:

poor wound healing — regional intestinal ulcers, but low enough to permit a close to normal reproduction (Paper II). But, the current tendency of an increasing prevalence of severe intestinal ulcers, especially in young grey seals (Paper II), points towards additional factors. In that connection the increasing environmental concentrations of polybrominated compounds (Sellström *et al.* 1993) should be seriously considered.

Parasites, fish and seals

Necropsy findings show that almost all of the Baltic grey seals are infested with hookworms. The prevalence of these parasites among Baltic seals previous to the severe environmental pollution is not known. In a British material of 46 Atlantic grey seals hookworms were found in 81% of the cases (Baker, 1987). No ulceration of the intestinal mucosa was found in the British material (Baker, 1987). The high frequency of hookworms associated with intestinal ulcers in the Baltic seals may be accidental, but it is more probable that the lesion incurred is facilitated by a defect immune system. The low levels of contaminants present in Atlantic (Law, Allchin & Harwood, 1989) and the high levels in Baltic seals (Blomkvist et al., 1992) are then taken into account. The finding in parasitology (Chapter 3.13.3) of ova of two liver flukes sp. (*Pseudamphistomum truncatum* and *Opistorchis* sp.) from the intestine of one grey seal (necropsied in 1996) is interesting. Later during this study, chronic cholangitis combined with hepatitis and abscess-like liver lesions have been found to be associated with infection with *Pseudamphistomum truncatum*. The frequency of such lesions has increased in recent years (Bäcklin B.-M., Swedish Museum of Natural History, personal communication). The aetiology of the chronic cholangitis in the harbour seal described in passage 3.10.4 is not confirmed but an effect of trematode seems probable.

It is not possible to prove a decreased immune response with the available necropsy material from Baltic seals. The increased incidence during recent years of verminous chronic cholangitis and hepatitis in grey seals of the Baltic and the still high prevalence of intestinal ulcers in animals of the same population (Bäcklin, personal communication) is thus unexpected when considering that the levels of harmful pollutants in the Baltic have gradually decreased since the middle of the 1970-ties (Bignert *et al.*, 2004). However, in addition to decreased immunosupressive effect caused by harmful pollutants a decreased exposure to such pollutants might also result in increased virulence of harmful parasites which might hamper an improvement of the health status of the seals. This would emphazice the importance of further study of these factors.

Bacterial infection - a significant factor ?

The bacteriological infections do not represent a main thread or key to the pathological findings. Based on the results obtained, the author's opinion is that bacterial infection probably is not a primary cause of the uterine lesions observed in Baltic grey and ringed seals, but that these infections are secondary to lesions associated with abortions or foetal death. Following the same assumption the intestinal bacterial infections are likely to be secondary to hookworm infections.

Phocine distemper epizootics in Northwestern Europe 1988 and 2002

The presence of changes of the BSDC in some grey seals raises the question whether the outbreak and course of the epizootic was influenced by environmental pollution. This could be a tempting explanation of the outbreak of the disease but there are several reasons to believe that this was not the case. The epizootic in Swedish waters was mainly located to the west coast where the contamination of the marine environment is much lower than in the Baltic, where only a minor number of animals in the southern parts were affected. The disease did not spread to the northern parts of the Baltic where the contamination is higher. The disease affected also younger animals whereas BSDC is prominent in older animals. There were no signs of BSDC among the harbour seals from the Baltic. Finally, the epizootic due to canine distemper viral (CDV) infection in Baikal seals affected a seal population, which had comparatively low tissue concentrations or organochlorines (Bobovnikova *et al*., 1989).

5. Summary and conclusion

A severe decline of the populations of grey and ringed seals in the Baltic took place during the second half of the 1900s. It was suggested that the cause was the contamination of the Baltic by industrial chemicals, above all organochlorines such as PCBs and DDT. High concentrations of these substances were found in the Baltic biota.

The author has performed necropsy or examination of organ samples from animals along the Swedish coast that were found dead on shore, by caught at fishery, or killed by hunting during 1977-2002. Reference material has been collected during hunting expeditions to Kings Bay in Svalbard and Helmsdale in Scotland. Colleagues at the Swedish Museum of Natural History in Stockholm and at the National Veterinary Institute in Uppsala have made analyses on concentrations of contaminants in the seals and in other Baltic species. Microbiological examinations were made at the last mentioned institute and at the University of Agricultural Sciences in Uppsala.

Multiple organ lesions were found most prominent in female reproductive organs, intestine and adrenals. Other serious changes were present in the integument, skeleton and kidneys. The disease picture was regularly occurring and characteristic and was named the Baltic Seal Disease Complex (BSDC).

Occlusions and stenoses of the uterine horns were the most prominent changes in the female reproductive organs, in some cases in connection with retained foetus and/or foetal membranes, findings that indicate that reproductive failure is an important factor behind the decline in seal populations.

Adrenocortical hyperplasia was a striking component of the BSDC. It was initially suggested in this investigation that an endocrine imbalance was associated with the disease complex. But, previous or ongoing inflammatory processes could also be implicated:
Adrenocortical hyperplasia is a common feature of prolonged stress in animals and man. The animals in this investigation have suffered from severe inflammatory processes in many cases in connection with advanced malnutrition due to both hampered ingestion and digestion of food, which could explain a stress reaction. This is in the author’s present opinion the most probable explanation of the adrenal changes and is based on findings during 1997-2002.

Inflammatory lesions were most prominent in the uterus and intestine. Deep ulceration of the colon was common, in several cases fatal ones leading to perforation of the intestinal wall. Other common sites of inflammation included the integument with lesions of claws, and masticatory bones.

Bacteriological investigation showed growth of different opportunistic or pathogenic micro-organisms at the sites of inflammation, but no regular findings which could be considered as the primary cause of the lesions. This may be interpreted as a sign of a defective immune response in the animals. Minor lesions in the mucous membrane of the ileocaeco-colonic region caused by hookworms are regarded as the primary event of the ulcerous processes facilitating the establishment of secondary bacterial infections.

The renal changes are more difficult to explain: Similar glomerular changes are seen in domestic animals and man but none of the etiological factors, which are present in these species, can be implicated in the seals. The tubular cell proliferations seem unique. No similar changes have been described previously to the author's knowledge.

A lower prevalence and severity of the lesions of BSDC has been demonstrated concurrent with a decreased contamination of the Baltic biota towards the end of the 1900s. This observation is a strong indication of the role of pollutants as the main factor behind the BSDC. Other factors may also be involved, however, as indicated by the observation that the prevalence of intestinal ulcers has not decreased.

At present there is an increasing understanding both regionally and globally of the risks following hazardous pollution by industrial chemicals, why it seems to be a realistic hope that a further recovery of the seal populations in the Baltic will take place in the future. Primarily, a deepened collaboration is wanted between the Baltic States to reduce the contamination.

A comprehensive material has been collected during the course of this investigation. Especially, a large histological material remains to be examined. Primarily, the lesions occurring in the intestinal ileocaeco-colonic region and the liver lesions caused by trematodes should be described further.

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English, Swedish and scientific names of higher animal species mentioned in the text:

Mammals:		
American mink	*Mustela vison*	Mink
Arctic ringed seal	*Phoca hispida*	Arktisk vikare
Baltic Ringed seal	*Phoca hispida botnica*	Östersjövikare
European ferret	*Mustela putorius furo*	Iller, frett
Grey seal	*Halichoerus grypus*	Gråsäl
Harbour or common seal	*Phoca vitulina*	Knubbsäl
Birds:		
Guillemot	*Uria aalge*	Sillgrissla
White-tailed sea eagle	*Haliaeetus albicilla*	Havsörn
Fish:		
Blenny or eelpout	*Zoarces viviparus*	Tånglake
Cod	*Gadus morhua*	Torsk
Dab	*Pleuronectes limanda*	Sandskädda
Eel	*Anguilla anguilla*	Ål
European sculpin	*Myoxocephalus scorpius*	Rötsimpa
Fourhorn sculpin	*Triglopsis quadricornis*	Hornsimpa
Flounder	*Platichthys flesus*	Skrubbskädda
Haddock	*Melanogrammus aeglefinus*	Kolja
Herring	*Clupea harengus*	Sill, strömming
Lemon sole	*Microstomus kitt*	Bergskädda
Long rough dab	*Hippoglossoides platessoides*	Lerskädda
Lucky proach	*Taurulus bubalis*	Oxsimpa, dvärgsimpa
Mackerel	*Scomber scombrus*	Makrill
Norway pout	*Trisopterus esmarkii*	Vitlinglyra
Perch	*Perca fluviatilis*	Abborre
Pike	*Esox lucius*	Gädda
Pikeperch	*Sander lucioperca*	Gös
Plaice	*Pleuronectes platessa*	Rödspätta, rödspotta
Poor cod	*Trisopterus minutus*	Glyskolja
Roach	*Rutilus rutilus*	Mört
Ruffe	*Gymnocephalus cernuus*	Gärs
Salmon	*Salmo salar*	Lax
Sandeels	*Ammodytes spp.*	Tobisfiskar
Sea trout	*Salmo trutta*	Havslaxöring
Smelt	*Osmerus eperlanus*	Nors
Sprat	*Sprattus sprattus*	Skarpsill, vassbuk
Two-spotted goby	*Gobiusculus flavescens*	Sjustrålig smörbult
Turbot	*Psetta maxima*	Piggvar
Vendace	*Coregunus albula*	Siklöja
Whitefish	*Coregonus oxyrhinchus*	Sik
Whiting	*Merlangius merlangus*	Vitling
Crustaceans:		
Opossum shrimp	*Mysis relicta*	Pungräka
Sea slater sp.	*Saduria entomon*	Skorv
Molluscs:		
Common mussel	*Mytilis edulis*	Båmussla

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