Marine Habitat Committee

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REPORT OF THE

Working Group on Marine Mammal Habitats

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8–12 March 1999

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

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1 INTRODUCTION

The meeting was held from 8–12 March 1999 at the ICES Headquarters in Copenhagen, Denmark, with Arne Bjørge (Norway) as Chair. The Chair welcomed the participants to the second meeting of the Working Group on Marine Mammal Habitats (WGMMHA) and summarised the meeting schedule. The ICES Environment Adviser, Janet Pawlak, welcomed the Working Group to ICES and the practical arrangements and facilities available at the ICES Secretariat were presented. The list of participants is attached as Annex 1.

2 APPOINTMENT OF RAPPORTEURS

Krystal Tolley and other members of the working group assisted the Chair as rapporteurs.

3 TERMS OF REFERENCE AND AGENDA

3.1 Terms of Reference

Terms of reference (ToRs) for the meeting, as given in ICES C.Res.1998/2:35, were to:

a) review progress in studies of marine mammal habitat requirements, including spatial and temporal aspects of habitat use, with emphasis on topics of relevance for marine mammal exposure to contaminants;

b) develop detailed plans for research on cause-effect relationships between contaminants and population-level effects in three species of seals—close cooperation will be sought with parallel research on three species of odontocetes as proposed by the IWC Scientific Committee;

c) review invited papers and other available documents on status, recent achievements, and new ideas for progress in techniques and methodology for life history studies, and define specific research projects and explore possibilities for their funding;

d) develop a scientific basis to handle the standing request from HELCOM for a triennial review of the health status of and effects of contaminants on marine mammals in the Baltic Sea;

e) review habitat-related transmission of parasites to marine mammals, including the threat presented by parasites to marine mammal health and the role of marine mammals as vectors in the distribution of parasites;

f) contribute to the ICES strategic planning process through assisting the Marine Habitat Committee in the following tasks:

   i. formulating tactics to achieve the six objectives adopted by the Committee,

   ii. suggesting and/or developing activities and products to fulfil the objectives,

   iii. estimating the resources required for each activity according to categories that will be supplied.

WGMMHA will report to ACME before its May/June 1999 meeting, and to the Marine Habitat Committee at the 1999 Annual Science Conference.

Justifications:

a)–c) These items fall under the remit of the ICES Five-Year Science Plan.

d) This item is based on a recurrent (triennial) request from HELCOM within the remit of WGMMHA.

e) This issue is of importance in several ICES areas, particularly in the Northwest Atlantic.

f) This is a request from the Marine Habitat Committee.

3.2 Adoption of Agenda

The agenda was discussed in light of the Terms of Reference and available documents for this meeting, and adopted as amended in Annex 2. The Working Papers and For Information Papers are listed in Annex 3.

4 REVIEW OF PROGRESS IN STUDIES OF MARINE MAMMAL HABITAT REQUIREMENTS

4.1 Habitat Use

Working Papers 4, 8, 9 and 15 provided new information on marine mammal habitat use, and were reviewed and discussed. WP15 was also referred for further discussion by the Working Group on Marine Mammal Population Dynamics and Trophic Interactions (WGMMPD) due to its relevance and importance to that working group.

4.1.1 Harbour seals

Site fidelity in harbour seals (*Phoca vitulina)* was found to be more pronounced than previously reported (WP8). Although some females migrate as juveniles, they return to their native locality to breed. Adult males spend relatively more time at localities up to 10 km away, but still exhibit strong site fidelity. The discrepancy with earlier estimates is probably caused by the short-term nature and/or low rate of recoveries of earlier marking techniques. Strong site fidelity was found to have consequences on harbour seal epidemiology, genetics, population biology and ecology, and is in concordance with recent observations from these fields. In addition, conglomerates of harbour seal colonies will act as a single population in some contexts and as a meta-population in others (WP8).

On the west coast of Sweden, harbour seals are assumed to be responsible for damage to eel traps, resulting in loss of catch and damage to gear (WP4). Therefore, harbour seal preference for various fish species was investigated near a seal colony in the northern Skagerrak. Seals were offered several different species of dead fish placed in traps similar to the eel traps used by fishermen. Seals preferred herring *Clupea harengus*, codfishes (Gadidae), and flatfishes (Pleuronectidae), while eel *Anguilla anguilla* and eelpout *Zoarces viviparus* were almost always rejected. Five-bearded rockling *Ciliata mustela*, bullrout *Myoxocephalus scorpius* and small labrids (Labridae) were never taken by the seals. Seal visits to the cages accounted for only 28 % of the feeding opportunities provided. This study also indicated that prey selection by harbour seals may be individualistic (WP4).

WGMMHA noted the new information on demographic and spatial organisation of harbour seal populations provided in WP8 and advised that movements and habitat use must be taken into account in experimental design when questions related to interactions with fisheries are pursued. WP8 clearly demonstrated that surveys and selection of study areas for sampling would be impacted by the complex spatial movements associated with gender, season, age, prey availability, and prey selection.

4.1.2 Ringed seals

The movements and diving behaviour of satellite-tagged adult ringed seals (*Phoca hispida botnica*) in Estonian coastal waters were monitored up to ten months, covering the period from moult to moult (WP9). Movements of adult animals from the Bothnian Bay in the northern Baltic were recorded for comparison. Adult ringed seals appeared to be stationary within regions and returned repeatedly to certain areas. Seals dived to the deepest bottoms (about 100 m) during summer and late autumn, whereas considerably shallower dives were recorded in winter. Both the mean duration and the mean depth of dives peaked in June and July, whereas a majority of dives were shallow and short during the remaining part of the year. Seals spent about 70 % of their time diving in July compared with 30 % in January–March. An independent measure of time spent at surface showed a similar flux, as seals spent 10–15 % of their time at surface during June and July compared to 35–50 % in October to March. In females 1–5 % of dives exceeded 10 min., while male dives showed larger variation as up to 10 % of dives were longer than 10 min. in July and August. Fewer than one percent of dives lasted for more than 25 minutes. Ringed seals double their weight during the period from May to October and a preliminary analysis indicates that the activity patterns are correlated to the occurrence of Baltic herring (*Clupea harengus*), but also linked to the timing of the reproductive cycle and moult (WP9).

This study presented in WP9 further demonstrated the importance of careful consideration of information on distribution and behaviour when designing research projects. WGMMHA therefore made recommendations on the methodologies which may be used in gathering such information. These recommendations are presented in Section 4.4.

4.2 Overview of Methods for Studies of Habitat Use based on Identifying and Tracking Individuals in a Population

4.2.1 Freeze branding

Some specific circumstances must be considered when designing surveys and sampling programmes of age- and sex-structured populations. The success of such projects is dependent on the degree to which high quality data can be collected on population growth rate, fecundity, mortality and migration. Difficulties arise because different age and sex classes often exhibit different behaviour, which makes them more or less likely to be sampled. This will result in systematically biased estimates of vital population parameters. The technique of freeze branding provides one possibility to study the behaviour of individual seals and thereby different segments of the population. The major benefits with this method are:

a) As brands are permanent, changes in the behaviour of individual seals can be detected as they pass through different phases in their life cycles.

b) As pups of the year are branded, the age of all branded animals is known.

c) Behavioural traits and life history variables of older animals can be linked to body conditions collected at the time of catching.

The disadvantages are:

a) Data on branded animals can only be achieved when they haul out.

b) An intensive observation effort is required for getting high quality data.

c) As the potential area of dispersal has to be covered by observations, a large effort is required in areas where the pup dispersal rate is high.

This technique may be very relevant for studies of habitat use, life history parameters and the spatial structure of resident seal populations.

4.2.2 Satellite transmitters

Recent advances in satellite transmitter tags have made it possible to obtain data on movements and diving behaviour of seals up to 10 months of the year (moult to moult) (e.g., WP9; Folkow *et al*,. 1996). Because of their small size, these tags can also be deployed on juveniles. Data obtained with this technique are essential for the construction of models on population energetics, when combined with information on diet and body condition parameters.

Advantages:

a) Detailed data on the movements of individual animals can be recorded for a major proportion of the year, which makes it possible to describe the short-term dynamics of populations.

b) The diving behaviour of individual seals can be studied in detail.

Disadvantages:

a) High costs. The price of one transmitter is about 5000 USD plus satellite time and data processing.

b) A general drawback is that transmitters must be glued to the fur of seals which enables them to be operative only until the annual moult when the transmitters are shed (or attached to dorsal fins or ridges of cetaceans which may result in even much shorter tracking times). This renders it impossible to follow specific animals for more than one year and therefore is less adequate in long-term studies of life-history parameters.

4.2.3 VHF tagging and tracking

The application of VHF telemetry in seal studies offers good opportunities to investigate individual behavioural patterns and time budgets. Haul-out behaviour, dispersal, dive behaviour, and dive-frequency/duration are specific aspects that can be studied in detail.

Another spin-off in the study of haul-out behaviour is the opportunity to carry out maximum likelihood population size estimations via mark-recapture experiments. The advantages of the VHF telemetry application compared to satellite telemetry are that the tag is relatively small, lightweight and inexpensive. The disadvantages are that depending upon the height of the antenna, the receiving range is restricted. There is generally no data logger connected, so continuous tracking is possible only when transmitters are within the receiving range of mobile or fixed automatic stations, making locating animals more complicated. VHF transmitters are not suitable for studies of life history parameters (cf. satellite transmitters above).

4.2.4 Data loggers

Using data loggers including a compass, speed-meter and depth-time-recorder gives the possibility to obtain detailed information about the activity of the animals. This technique has been successfully used on several animals including seals in the Wadden Sea. Disadvantages are that the tag needs to be recovered, and the loggers are therefore at the moment used in combination with satellite- and VHF-tags. However, techniques are under development to transfer logged data via satellite. Furthermore, hydrodynamic saddle-packs have been developed to reduce drag and minimise interference in diving performance.

4.2.5 Hat tags

The need for the identification of individuals from a distance, in particular for mark-recapture studies, has led to the development of a new cheap, brightly coloured, head-mounted tag for pinnipeds on which a large identification code is embossed (Hall *et al.*, submitted). Plastic, extrusion moulded pyramidal tags designed by the Sea Mammal Research Unit (UK) were initially produced for use on post-weaned grey seal pups but have been used on rehabilitated grey seals from rescue centres and female harbour seals in the US. The tag is made from high impact styrene, which is strong enough to withstand abrasion. It can be made in a variety of bright, photo-stable colours and has a large two-letter or number cypher embossed onto its sides. Further information (such as a contact telephone number for finders of shed tags) can also be added. The tag is filled with a resin filler mixture of polyester casting resin and microspheres, which make it buoyant and imperious to pressure. The tag weighs 30 g. The tags are glued to the fur of animals using quick-setting epoxy resin. The tags are lost when the animals moult and will float.

4.2.6 Photo identification

Photo-identification techniques have been used for many years for the identification of individual cetaceans, using fluke and fin marks. A technique was then developed for use in grey seal females (Hiby, 1995) whereby the markings on the pelage around the face and neck are used as 'fingerprints' for the identification of individuals. A standardised area of pelage is extracted from the photographs obtained and a system is used which compensates for the different angle of photographs, and the position and head shape of each individual. A computerised visual pattern-matching program was developed which can store thousands of these images. They are then used as a database of 'marked' individuals for comparison with new images that are collected in the field. The results from such studies can be used in mark-recapture models for estimating abundance and survival. This system is now being extended with new models for post-weaned grey seal pups that may retain some of their markings into adulthood. If this is the case, particularly for females, then results can be used for studying the long-term recruitment to the adult population and survivorship beyond age one. A photo-identification system is also being developed for use on harbour seals and for use with a specially adapted camera attached to a telescope so that animals can be successfully photographed from much longer distances than is currently possible with standard photographic lenses. Because of the different nature of the patterns on the pelage of different seal species, specific models must be developed for each species. This technique can be very powerful for marking large numbers of animals with minimal disturbance to individuals.

4.2.7 DNA fingerprinting

Automated analysis and comparison techniques in the field of molecular genetics mean it is now feasible to process large numbers of samples and thus obtain unique individual genetic identifiers (DNA fingerprints) and match them to individuals. Suitable tissue samples can be obtained from living animals, thereby permitting longitudinal studies. There is a considerable body of literature, primarily using photo-identification techniques, on the use of individual recognition data from marine mammals to estimate biological parameters, population size, movements and stock identification. The applicability of this approach, including the establishment of a centralised catalogue, should be examined for seal populations.

4.2.8 Transponders

Microchips (transponders) are injected subcutaneously in order to identify an individual all its life by reading an identification code in the microchip with a scanner. This method is inexpensive and has been used on seals in the Wadden Sea, Isle of May, Moray Firth and the Baltic. With the current technology the animal needs to be approached closely in order to be able to read the transponder, but new techniques are under development to enable reading the identification code from a distance (e.g., from an airplane).

Animals can be equipped during wild-capture or rehabilitation. It is important that the site of implantation is standardised and that a standard scanning system be used. Scanning should also be performed prior to dissection of by-caught and stranded animals. This technique could also be of use during feeding experiments with wild animals such as in the study conducted in the Skagerrak (WP4).

4.3 Ethics and Effects of Study Techniques on Animals

All techniques for marking or tracking animals have implications for the health and/or behaviour of marked animals. These implications may be viewed from two directions: a) the effects on study results and b) the effects on animal health. In most countries, an independent review of the ethics of a study is carried out before it proceeds. The aim of this review is to avoid unacceptable impacts on study animals. More subtle effects though may not be reviewed in the process. It is important that potential effects on animal behaviour be evaluated in studies that attach items to animals. There have been rather few such studies so far, but results from them indicate that effects may be large. Wilson *et al.* (1986) found that a relatively small radio tag attached to the back of a penguin increased its oxygen demand by 30 % over that of an untagged animal, and that diving behaviour was greatly modified. Without such impact studies, there is a risk that the results from tagging studies may be invalid due to changes in individual behaviour.

4.4 Recommendations

When considering the methods available for individual recognition and tracking of animals, WGMMHA felt that some of the traditional techniques have potential for application in combination with the newly developed techniques. Freeze branding is a technique that provides a 'tag' of life-long duration in seals and is well suited for habitat use and life history studies. In combination with temporary tracking by signal-transmitting tags or data loggers that provide detailed information in a shorter 'window' of time, this technique may provide new information to understand the ecological and social role of individual animals in a population.

In the studies of harbour seals in the Skagerrak (WP8), no additional mortality or abnormal behaviour of freeze-branded seals was observed. WGMMHA therefore **recommended** further studies using freeze branding in combination with other tracking techniques to reveal the spatial aspects of pinniped ecology.

WGMMHA noted that the photo-identification technique developed for grey seals (Hiby, 1995) may have potential to follow post-weaned grey seals until they are recruited into the population of adult seals. WGMMHA **recommended** further development of this method also with regard to other species.

Further, WGMMHA pointed to the value of study populations where a large proportion of the animals are individually identified, and **recommended** that this photo-identification technique or the freeze-branding technique be applied in populations where other detailed monitoring programmes are conducted (cf. Agenda Item 5.7).

5 PROPOSAL FOR RESEARCH ON CAUSE-EFFECT RELATIONSHIPS BETWEEN CONTAMINANTS AND POPULATION-LEVEL EFFECTS IN PINNIPEDS

5.1 Introduction

Many studies have established an association between the exposure of marine mammals to persistent environmental contaminants and a variety of detrimental physiological effects. However, these findings have been unable to provide definitive evidence for cause-effect relationships or determine how physiological changes at the individual level translate to effects on populations. In 1998 WGMMHA reviewed the available information on population-level effects of contaminants in marine mammals. The toxicity of different compounds was considered and the polyhalogenated organic compounds (PHOCs), including the 'classical' polychlorinated biphenyls (PCBs), were focused on. Generally the non-*ortho* PCB congeners were considered more toxic to most systems investigated than the mono-*ortho* congeners, with variability between congeners and between species exposed (Battershill, 1994). Effects on terrestrial mammals have been demonstrated, and there is evidence to suggest that marine mammals have a smaller capacity to metabolise chlorobiphenyl (CB) congeners than terrestrial mammals (Tanabe *et al.*, 1988; Kannan *et al.*, 1989). They are therefore potentially more vulnerable than terrestrial mammals to the toxic effects of PCBs in general, and to the planar and coplanar PCBs in particular. The Working Group concluded that CBs are likely to affect the health of certain marine mammal populations, but the extent of this effect is unclear, despite some experiments linking contaminants to their sub-cellular, cellular or systemic level effects (De Guise *et al.*, 1995b, 1995c; DeSwart *et al.*, 1994, 1996; Ross *et al.*, 1995, 1996). Although suppression of population growth and fecundity rates have been reported for marine mammal populations resident in contaminated areas (e.g., grey and ringed seals in the Baltic Saa, harbour seals in the Wadden Sea) there is no well-defined cause-effect relationship linking specific contaminants to population-level effects. In 1998, the Working Group therefore recommended development of a research programme aimed at understanding and describing the causal relationships between environmental contaminants and population-level effects in marine mammals.

Recognising plans developed by the Scientific Committee of the International Whaling Commission (IWC, 1995, 1997) for research on the effects of contaminants in cetaceans, WGMMHA focused on effects on seals and complementary problems that may facilitate collaboration between the planned research efforts. Below is an edited synopsis of the findings that led WGMMHA to recommend a research programme.

The most commonly reported responses to CB exposure, largely based on data from laboratory studies, indicate that a range of body systems may be affected. These include body weight loss, thymic atrophy, impairment of immune responses, hepatotoxicity and prophyria, chloracne and related dermal lesions, tissue-specific hypo- and hyperplastic responses, carcinogenesis, teratogenicity and reproductive toxicity (Amdur *et al.*, 1991). Hallmarks of exposure are the induction of both phase I and phase II drug metabolising enzymes and it is the induction of the phase I, P450 enzymes in particular (recently reported in various cetaceans and seal species), in conjunction with higher PCB uptake which increases the concern about the toxicological importance of the non- and mono-*ortho* CB congeners for vulnerable and threatened marine mammal species (Norstrom *et al.*, 1992; Stegeman and Hahn, 1994; White *et al.*, 1994). Many studies have measured the concentrations of mono- and non-*ortho* CBs in marine mammal tissues (Tanabe *et al.*, 1987; Green *et al.*, 1996; Oehme *et al.*, 1995a, 1995b; Nakata *et al.*, 1995; Koistinen *et al.*, 1997; Kuehl *et al.*, 1994; Lake *et al.*, 1995; Corsolini *et al.*, 1995) but little information exists on their specific effects.

The impacts of non-*ortho* CBs and mono-*ortho* CBs on the reproductive system and early development appear to have a strong biological effect on marine mammal populations. Experimental reproductive failure in harbour seals was induced by feeding seals on fish from a polluted area of the Wadden Sea (Reijnders, 1986). Lesions of the reproductive system, such as stenosis and occlusion of the uterus, have been described for seals from British, Swedish and Finnish waters (Helle *et al.*, 1976a, 1976b, 1980; Baker, 1989; Olsson *et al.*, 1994; Bergman, 1997). These lesions have been attributed to high PCB and DDT levels in these animals. Between 1977 and 1986, about 42 % of four-year old female Baltic grey seals that were examined showed stenosis or occlusions of the uterus, whereas between 1987 and 1996 only 11 % had those lesions. The pregnancy rate during the first period was only 17 %, whereas in the second period it increased up to 60 % (Bergman, 1997). It has been suggested that an improvement of the seal population is due to a declining contaminant burden in Baltic biota (ICES, 1997).

Evidence of the effects of CBs on the immune system has been demonstrated in both humans and other animals. In the Netherlands, seals experimentally fed on contaminated fish showed functional failure of Natural Killer (NK) cell activity and a decrease of T-cell function with increasing polyhalogenated hydrocarbon concentrations in prey in comparison to seals fed on less-contaminated fish from the Atlantic (De Swart *et al.*, 1994, 1996; Ross *et al.*, 1995, 1996). These investigations support suggestions that CBs reduce resistance to cancer development (De Guise *et al.*, 1995a) and to morbillivirus infections (Dietz *et al.*, 1989; Kannan *et al.*, 1993; Aguilar and Borrell, 1994). Other experimental and empirical studies could not reproduce such effects on mortality (Hall *et al.*, 1992; Harder *et al.*, 1992). However, morbilliviruses are highly virulent agents also acting immunosuppressively, so it is therefore very difficult to investigate the potential relationship between CBs and epizootics.

Several authors have described the loss of bone substance in the skull and asymmetry of the skull for several seal species from the North and Baltic Seas (Stede and Stede, 1990; Zakharov and Yablokov, 1990; Bergman *et al.*, 1992; Mortensen *et al.*, 1992; Olsson *et al.*, 1994). Swedish investigations suggest that lesions of the skeletal system were related to hyperplasia of the cortex of the adrenal gland, caused by a high load of organochlorines (Bergman and Olsson, 1986; Bergmann, 1997). This is supported by a new study on harp seal pups. Those animals fed on increasing doses of specific PCB congeners showed higher cortisol levels which may indicate an adrenal hyperplasia in response to PCB-exposure (Lohman *et al.*, 1998). Further changes of the endocrine system associated with the CB levels are described for the thyroid gland. Harbour seals fed on high concentrations of PCBs showed decreased concentrations of plasma retinol (vitamin A) and thyroid hormones in comparison to another group fed on less-polluted fish (Brouwer *et al.*, 1989).

A higher prevalence of tumours such as leiomyoma is also described for the Baltic grey seal (Bergman, 1997). About 53 % of females investigated between 1977–1986 showed leiomyomas, whereas between 1987–1996 only 44 % carried these benign tumours, which in some cases may hamper reproductive success.

The 1998 recommendation of WGMMHA was endorsed by the ICES Council, and at the present meeting, a proposal was developed for research on cause-effect relationships between contaminants and population-level effects in three species of seals with populations resident in a gradient from polluted to relatively pristine environments. The planned project is composed of several sub-units that will contribute to the development of a conceptual predictive model. This model will, through several steps, link the contaminants in the environment with population-level effects in marine mammals (Figure 1). Contaminants are transferred from the environment to marine mammals via prey species. Fish are important prey species to seals, and fish have little capacity to metabolise many pollutants, such as the organochlorine compounds. Thus, they are carriers of these persistent chemicals, and efficient vehicles for transferring contaminants to seals. Understanding the temporal and spatial distribution of seals and their prey species is therefore a fundamental first step in this process.

The plans given below describe a core programme that provides the minimum amount of research that should be conducted to reach the defined objectives. An additional programme is also described. The additional programme will complement the core programme and provide the basis for a more holistic understanding of the problems involved, and facilitate evaluation of interspecific differences.

5.2 Objectives

5.2.1 General objective

To determine a conceptual predictive model linking tissue levels of pollutants in seals with effects on individuals and populations. This model will contribute to our understanding of the processes involved and will assist in mitigating and preventing anthropogenic impacts on the marine environment.

5.2.2 Specific aims

1) To develop and expand biomarkers indicative of physiological responses of animals to contaminant exposure in controlled experiments. These developments will be applied to wild populations which occur over a gradient of pollution.

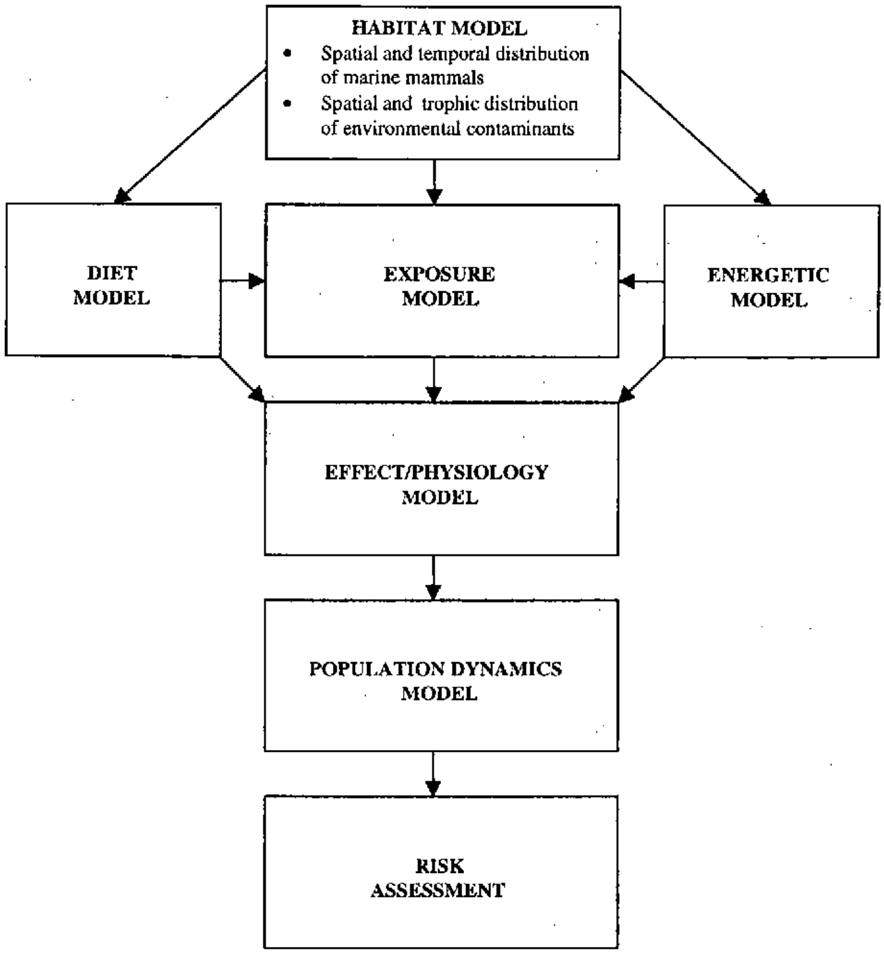
2) To begin the process of developing a modelling framework to describe processes linking tissue pollutant levels in individuals to population-level effects.

5.3 Compounds of Relevance to Toxic Effects in Seals

5.3.1 Compound packages recommended for inclusion in the analysis

Different classes of polyhalogenated organic compounds (PHOCs) are generally considered to be highly resistant to environmental degradation processes such as photo-oxidation and enzymatic degradation; they are also highly lipophilic. As a consequence, they are likely to bioaccumulate in biota. Biomagnification is especially strong from water-breathing prey to air-breathing predators. Because of their persistent nature, they can also be transported over large distances. The packages of compounds recommended for inclusion in the study are shown in Table 1.

Figure 1. A general outline of the framework in which the planned research will be carried out.



5.3.2 Argumentation for the choice of the compound classes proposed for study

Package 1: 'Classical' organochlorines. All these classes of compounds are presently banned in the Western world, but they still occur globally in the residues of marine mammals. These compounds are analysed on a routine basis in most laboratories; analysis can be done easily and at a relatively low cost by gas chromatography with electron capture detection (GC-ECD). Moreover, the effects on the reproductive and the immune system that will be studied, might be caused by complex mixtures of contaminants including these classical groups as well as some less-studied classes of a more recent origin.

- Polychlorinated biphenyls (PCBs): Used as cooling fluid in transformers and capacitors, hydraulic oils in the mining and offshore industry, plastic softeners, NCR-paper, and in paints. Effects on the reproductive and immune system of seals in the Wadden Sea and the Baltic Sea have been largely attributed to the high levels of PCBs and DDTs, as well as some of their metabolites (discussed below). References on marine mammals: Reijnders, 1986; Ross *et al.*, 1996; WP16 and WP17.
- DDTs: An insecticide (4,4'-DDT is the parent compound). 4,4'-DDE and 4,4'-DDD are bioaccumulating metabolites. Minor amounts of 2,4'-DDT are in the technical mixture.
- Chlordane: An insecticide, and a successor of DDT which is banned in Western countries, but regularly occurs in residues of marine animals. Composition of technical mixtures (about 150 compounds) is heavily changed by environmental degradation processes. Some metabolites are stable (e.g., oxychlordane in polar bears at Svalbard). These compounds are endocrine disruptors and carcinogenic in microbial test systems.

Table 1. Packages in the Core Programme and Additional Programme, and tissues required. Non-invasive sampling (NI). Core Programme requires 10 animals per cell (same site; same sex; same age group; same tissue). Additional Programme requires 5 animals per cell. By-catch (BC) requires 5 animals per cell, and *in vivo* experiment (Exp.) requires 10 animals per age and sex-specific group.

	TISSUES		Prey
	Exp., NI	BC *	
Core Programme (all samples)			
Package 1: Classical OCs (PCBs, DDTs, chlordanes)	blood, blubber	blood, blubber, liver	muscle or liver
Package 2: Brominated flame retardants	blood, blubber	blood, blubber, liver	muscle or liver
Additional Programme (selected samples)			
Package 3: nonylphenols (inventory high-low)	blubber	blubber	muscle or liver
Package 4: organotins (inventory high-low)	-	liver	muscle or liver
Package 5: planar PCBs, PCDDs & PCDFs	blubber	blubber, liver	muscle or liver
Package 6: toxaphene	blubber	blubber	muscle or liver
Package 7: OH-PCBs	blood	blood	muscle or liver
Package 8: MSF-PCBs	blubber	blubber	muscle or liver
Package 9: trace metals	-	liver, kidney, skeleton	muscle or liver

*When by-caught animals are obtained within a few hours after death, liver samples should also be taken for biochemical purposes (preparation on site and freeze in liquid nitrogen).

When PCBs, DDTs, and chlordanes are analysed, the levels of the following two compounds are obtained more or less automatically. They have not been related to specific effects on marine mammals under field circumstances.

- Dieldrin: An insecticide and stable metabolite of the insecticide aldrin.
- Hexachlorobenzene (HCB): Has been used as a fungicide (mainly on seeds). It is also an industrial by-product, and is formed in waste incineration.

The classes of chemicals mentioned in packages 2–8 should all be analysed by gas chromatography with mass-spectrometric detection (GC-MS).

- Package 2: Brominated diphenylethers (PBDEs) and polybrominated biphenyls (PBBs) are two classes of flame retardants which are still widely used in, e.g., plastics of common household electronic equipment, PCs, car seats, and textiles. PBBs were the predecessors of the PBDEs. Mixtures with mainly tetra- and penta-compounds have been gradually replaced by highly brominated congeners, which are claimed not to bioaccumulate. These compounds have been found in residues of fish, as well as in marine mammals from the Baltic Sea, the North Sea, and recently in the blubber of live-stranded sperm whales, indicating that they have also reached open-ocean food chains. They have been found to be endocrine and immune system disruptors in laboratory rodents.
- Package 3: Nonylphenols. Used in the offshore industry (emulsifier) and stable metabolites from alkylbenzene-ethoxylates (surfactants). Strong endocrine disruptors by binding to the oestrogen receptor, causing, e.g., the feminization of male fish under field circumstances. There is no information about the levels in marine mammals yet. Because of their mechanism of action, inventory research of their levels is considered to be very important.
- Package 4: Organotins. Tributyltin is used as an active biocide in antifouling paints on ship-hulls. It is still legally used on sea-going ships >25 m. It has been found to cause imposex (growth of a penis homologue and a vas deferens) in female snails in coastal areas as well as open shelf seas throughout the world, and high concentrations have been observed also in marine mammals. Impairment of the immune system has been observed in laboratory studies with rats. Metabolites are di- and mono-butyltin. Triphenyltin is also added to some antifouling paints, but is also used as a fungicide in agriculture (potato crops). Because of the high concentrations observed in cetaceans and their effects on marine snails, inventory knowledge of their levels in seals is considered to be very important.
- Package 5: Planar PCBs, polychlorinated dibenzodioxins (PCDDs) and dibenzofurans (PCDFs). Planar PCBs are specific compounds present in PCB mixtures that lack chlorine substitution in the *ortho-* positions to the inter-ring bond. Because of this there is no strong steric hindrance for having both aromatic rings oriented in the same plane. These compounds have a toxicity profile that is equal to that of the PCDDs and PCDFs, which are by-products of

chlorinated waste incineration and some industrially synthesised compounds. They are known to be endocrine disruptors and promoters of carcinogenicity. Effects of these compounds in marine mammals cannot be separated from those of PCBs in general. In studies with mink, the fraction with the planar compounds was very potent.

- Package 6: Toxaphene. A complex insecticide mixture of about one thousand chlorinated bornanes, bornenes and borna-dienes. It was the successor of DDT in the USA where it was widely used until its ban in 1980 after which time its production was moved to Central America. Has been produced and probably also used in Eastern European countries, but never officially in Western Europe. Despite this, concentrations in cod from the northern North Sea are higher than those of the PCBs. Composition of technical mixtures is heavily changed by environmental degradation processes. It is thought to be an endocrine disruptor and has been found to be carcinogenic in microbial test systems and experiments with mammals. Addition of liver microsomes of harbour seal decreases genotoxicity in microbial system, i.e., biotransformation may be protective.
- Package 7: Hydroxy PCB metabolites. Stable hydroxy-metabolites interfere with the transport regulation of thyroid hormones and vitamin A. Significant differences in plasma levels of thyroxin and vitamin A occurred between seals exposed to polluted fish from the Wadden Sea and seals fed with clean fish from the Atlantic Ocean.
- Package 8: Methylsulfone PCB and DDE metabolites. Although derived from PCBs and DDTs, these compounds have been listed as a specific group because they have their own mechanisms of toxicity. Methylsulfone metabolites are also very stable and have been related to sterility by stenosis and occlusions (scars from miscarriages) in ringed seals.
- Package 9: Trace metals Cd, $(CH_3\text{-})Hg$, Pb, Cu, and Zn. Cd may cause reproductive effects. CH_3-Hg affects the central nervous system. In contrast to Cu and Zn, Cd, Hg, and Pb are non-essential metals. Levels of Se and metallothioneins should also be measured in this context, because they may protect against the toxicity of these metals by decreasing their bioavailability at target sites (antagonistic effect).
- Polyaromatic hydrocarbons (PAHs) have not been included in this programme as very few residues of the parent compounds are found in fish, because they rapidly metabolize PAHs. PAH residues were also low in fish-eating dolphins. In squid, residues may be present because biotransformation is less efficient in molluscs. PAHs are formed in combustion processes (unsubstituted compounds) and are present in oil (mainly alkylated compounds). Some representatives are strong carcinogens via the formation of adducts with DNA after biotransformation by the cytochrome P450 system.

5.4 Responses and Indicators of Toxicity (Biomarkers)

This section details the response variables and endpoints that should be used to assess reproductive, immune system and other disorders in pinnipeds. Some of these parameters have not been measured in seals and some validation or verification studies would be needed before they could be included in the range of responses being measured in the field (this includes assessment of baseline levels, inter-individual and inter-annual variation). If no data already exist, for the blood-related proteins and hormones in particular, the variability within individuals over at least one annual cycle is required to determine seasonality. Also baseline studies for the parameters of the immune system of live and dead animals are needed.

Exposure to environmental contaminants will first affect molecular and cellular processes. If the adaptation and repair mechanisms are overloaded, the effect will be transferred to another level (i.e., tissue, organ, individual, population, or community/ecosystem). Effects at higher hierarchical levels are always preceded by changes in 'lower' processes, allowing the development of early warning biomarker signals of effects at 'higher' response levels (Beyer, 1996).

The following criteria for biomarkers have been identified by Stegemann *et al.* (1992): (1) The assay to quantify the biomarker should be sensitive, reliable, and 'relatively' easy; (2) baseline data for the concentration/activity of the biomarker should be known in order to be able to distinguish between natural variability (noise) and contaminant-induced stress (signal); (3) the basic biology/physiology of the test organism should be known so that sources of uncontrolled variation (growth and development, reproduction, food sources) can be minimised; (4) all the factors, intrinsic as well as extrinsic, that affect the biomarker should be known; (5) it should be established whether changes in biomarker concentration are due to physiological acclimation or to genetic adaptation; and finally, (6) changed levels of the biomarker should be correlated with the 'health' or 'fitness' of the organism.

5.4.1 Effects on the immune system

The immune system, particularly in neonates and juveniles, is sensitive to effects of contaminant exposure in a dose-dependent way. Some parameters have already been established as possible indicators of immunotoxicity for use in the field but some new markers and reagents have become available which should be evaluated for their ability to determine immune malfunction in exposed animals. In addition, measures will be included that may allow the distinction between responses due to pathogens rather than contaminants to be made (Table 2).

Table 2. The minimum set of measures that should be used to assess immune function in wild seals in conjunction with validation in captive studies. Blood and blubber samples will be collected from all live animals captured in the field and will be submitted for core contaminant and indicator response analyses. Further examinations will be carried out when the appropriate tissue samples are available, e.g., immuno-histochemistry on organs from freshly dead animals.

Indicator	Age class of animals	Tissue/sample
CORE PROGRAMME		
Leukocyte counts	All	Blood
Differential white cell counts	All	Blood
Erythrocyte counts	All	Blood
Erythrocyte sedimentation rates	All	Blood
Acute phase proteins (CRP)	All	Blood
Thymic hormones	Juveniles/neonates	Blood
Cytokines (IL-2, IL-6)	All	Blood
Immunoglobulins (IgG, IgM)	All	Blood
Lymphocyte sub-populations using cell surface markers and flow cytometry	All	Blood
Lymphocyte proliferation tests	All	Blood
Natural killer cell activity	All	Blood
ADDITIONAL INDICATORS		
Antibody responses to antigenic challenge	All	Blood
Immuno-histochemistry	All	Lymph nodes, thymus, spleen
Innate immune measures	Juveniles/neonates	Blood

Study of the functional and static innate and adaptive immune system indicators would be carried out using blood samples. Functional response assays might be more difficult to carry out in the field although it may be possible to devise 'mobile' laboratories to, for example, cryopreserve lymphocytes in the field, just after blood samples have been obtained, so that the more detailed studies and assays could be carried out in the 'static' laboratory.

Static responses would include: immunoglobin concentrations (IgG and IgM), lymphocyte counts and differential counts, erythrocyte counts, erythrocyte sedimentation rates, acute phase proteins, thymic hormones and cytokine concentrations. Comparing levels of lymphocyte sub-populations would be measured using appropriate reagents and marine mammal-specific polyclonal or monoclonal antibodies (e.g. markers for CD4 and CD8 T-cell surface monocyte and B cell markers).

Functional responses would include: lymphocyte proliferation tests, neutrophil phagocytosis and respiratory burst and NK activity. Responses to non-pathogenic antigens and lectins can also be measured in both captive and wild animals.

Innate and/or adaptive immunity: Very little is known about some aspects of the innate immune system in marine mammals (this is the very basic level of defence, comprising white cells, cytokines and peptides and acute phase proteins) and it should be investigated further, since it might be a very important primary arm of the immune system. Immunohistochemistry would be carried out on samples of spleen, lymph nodes and thymus from dead animals.

5.4.2 Effects on reproduction and early development

Areas where the effects of exposure to environmental contaminants have been found in seals are on reproduction and early development. Endocrine disorders are a common denominator for many of these effects. In order to put disorders into perspective it is essential to obtain further baseline levels, particularly from studies in captive animals. One of the areas we focus on is to establish hormonal profiles throughout the cycle. Studies on animals in captivity which are fed fish from both clean and contaminated sites will allow us to determine effects on male reproduction not previously investigated. These indicators are detailed in Table 3. The second set of indicators in the table is useful in understanding modes of action leading to hormonal imbalance and malfunctioning of the endocrine system. Thirdly, pathological diagnostic techniques will be used where organs are available from dead animals. Many of these are mainly aimed at establishing endpoints resulting from hormonal disorders and hormone disturbance in early development.

Table 3. A core set of indicators is shown. These represent the minimum requirements for the project. All indicators in the core set will be measured in the animals captured in the wild. Blood samples will be taken from live animals and all other samples will be collected from the available dead animals.

Indicator	Age class of animals	Tissue/sample
CORE PROGRAMME		
Testosterone	Adults and sub-adults	blood
Oestradiol	Adults and sub-adults	blood
Progesterone	Adults and sub-adults	blood
Vitamin A	Juveniles and neonates	blood
Thyroid hormones	Juveniles and neonates	blood
Oestrogen receptor binding capacity	Adults	ovary, liver, brain, blood
Glial fibrillary acidic proteins, synatophysins and nerve growth factor	Fetuses and neonates	Brain
Cytochrome P450 (CYP1A) important for oestradiol metabolism and CYP2B important for testosterone metabolism	Neonates, sub-adults and adults	liver, skin, blood
Testosterone hydroxylase assay	Adults and sub-adults	liver
ADDITIONAL INDICATORS WHEN TISSUES ARE AVAILABLE		
Spermatogenesis	Adults	testes
Female reproductive tracts	Adults	complete
hermaphrodism	Adults	reproductive organs

5.4.3 Effects on other systems and organs

Some other abnormalities that could not be directly associated with reproductive or immune system disorders, such as skull lesions and claw malformations, have been associated with environmental contaminant exposure in seals. These are generally associated with disease syndromes associated with hyper-adrenocorticism. Early signs of these malformations may be detected by changes in levels of calciothrope hormones (Table 4).

5.4.4 Biotransformation enzymes and other indicators of responses to exposure

By measuring the activity of the enzymes responsible for the detoxification of environmental contaminants, further indicators of exposure to a range of contaminants can be obtained. We will investigate the activity of these enzymes, which are also indicators of response to exposure, where fresh tissue samples are available from the study populations. We will also measure metallothionein, which is important for protection against effects of heavy metal toxicity (Table 5).

A general health screen using clinical chemistry and standard haematology will also be included as routine. Cytochromes P450 may be related to sex, age and species so the effect of these factors on activity levels will be investigated.

Table 4. Indicators to be used to determine the occurrence of abnormalities and early development.

Indicator	Age class of animals	Tissue/sample
CORE PROGRAMME		
Calciothrope hormones (e.g., calcitonin, vitamin D, parathormone, prolactin)	Adults and sub-adults	blood/urine
Enzymes and bone metabolites (e.g., bone alkaline phosphatase, tartrate resistant acid phosphatase)	Adults and sub-adults	blood
Calcium (total concentrations) using in particular double X-ray techniques	Adults and sub-adults	blood, bone
ADDITIONAL INDICATORS WHEN TISSUES ARE AVAILABLE		
Skull lesions	Adults	skull
Investigations on bone structure (e.g. morphology, histology, immunocytocology, HPLC, X-ray)	Adults	upper/lower jaw

Table 5. List of biotransformation enzymes and other indicators of response to exposure that will be included in the programme.

Indicator	Age class of animals	Tissue/sample
CORE PROGRAMME		
Metallothioneins	All	kidney/liver
ADDITIONAL INDICATORS WHEN TISSUES ARE AVAILABLE		
Phase I biotransformation, cytochromes P450 (alkoxyresorufin assays (EROD, PROD, etc.) western blotting (also included in Table 1)	Adults and sub-adults	liver
Aldrin epoxidase (CYP2B) also included in Table 1	Adults and sub-adults	liver
Phase-II conjugation enzymes, glutathion-*S*-transferases	Adults and sub-adults	liver
Porphyrins	Adults	liver
Luciferase	All	blood, blubber
TCDD bioassay	All	liver

5.5 Confounding Factors

Clearly there are many confounding factors that need to be considered in all studies (factors associated with the exposure and response which may account for any relationship found). They include age, sex and reproductive condition, nutrition, circumstances of sampling, population structure, geographic location, and season. Detailed information on the health status is required in order to try to distinguish effects of contaminants from effects caused by other stresses. For live animals (captivity, wild capture) this should include weight, length, girth, nutritional status, blood status, microbiology, serology (e.g., antibodies to morbillivirus) and parasitology as well as body temperature. For carcasses examination should be done according to the Proceedings of the first ECS workshop on cetacean pathology (Kuiken and Hartmann, 1993) including weight, length, girth, nutritional status. All organ systems should be examined in detail and as thoroughly as possible for any lesions. Depending on the macroscopic findings, further investigation on the histology, bacteriology, virology, parasitology, toxicology (algae) are necessary. Special investigations (e.g., electron microscopy) should be performed if required. This additional information must be collected for the findings of contaminant exposure and individual response to be properly interpreted.

5.6 Laboratory Experiments

5.6.1 *In vivo* experiments

Validation of indicators using studies on captive animals

Blood samples will be obtained regularly from individuals in semi-field conditions to determine individual variability and seasonality in reproductive hormones and immune parameters. Reproductive system studies using captive animals will enable annual changes in testosterone profiles, gonadotrophic hormones and thyroid hormones to be established, the latter particularly in young animals. For the immune system, the lymphocyte cryopreservation techniques will be validated for their applicability and use in the field. Isotopically labelled contaminants in prey fed to captive animals will enable partitioning and transformation studies to be carried out in conjunction with confirming the sensitivity of the battery of immune function assays to determine immunocompetence in individuals. Indicator PCB congeners including both recalcitrant and metabolisable congeners (for example, CB153, CB138, CB128) will be labelled with radioactive isotopes so that the time course of their uptake, distribution and metabolism can be determined within and between individuals. Regular blood and blubber samples taken from these individuals will be analysed for their contaminant content and profiles will be related to those in the prey eaten. Faecal samples will also be analysed for excreted congeners and metabolites.

Other systems under study include the skeletal system and studies on changes in calciothrope hormones with time should be conducted.

5.6.2 *In vitro* experiments

Several constituents of mixtures of organohalogen compounds, believed to be resistant to biodegradation in nature, were proven to be enzymatically metabolisable in *in vitro* assays carried out with microsomal preparations made from fresh marine mammal samples. It appears that considerable differences in metabolic capacity can exist, not only between the different classes of animals, but even between different species belonging to the same order. The central role with respect to the primary metabolic attack is played by the cytochrome P450 dependent mono-oxygenase enzyme system (CYP 450). CYP 450 is known to occur throughout the entire biological realm, from bacteria to mammals. More than 150 iso-enzymes belonging to 27 families have so far been isolated. Enzymes belonging to the families 1 to 4 are known to be involved in the biotransformation of xenobiotics. In higher animals, CYP 450 can be found in several organs, but the liver plays a central role in the metabolism of xenobiotics.

The research described in this proposal will establish:

1) The link between the capacity of different animal species for the biotransformation of a number of classes of contaminants to the expression of CYP 450 iso-enzymes of the families 1 to 4. For this purpose, *in vitro* biotransformation assays will be carried out with microsomal preparations of the different seal species. Individual compounds belonging to the contaminant classes chlorobornanes (toxaphene), chlordanes, polybrominated diphenyl ether flame retardants, polychlorinated biphenyls, polychlorinated dibenzodioxins and furans. When relevant, stereochemical aspects of biotransformation will also be investigated.

2) A link between the suitability of a compound as a substrate for the P450 system in the *in vitro* assays and its behaviour in the marine food chain involving fish and different seal species as derived from residue analyses of the compound classes mentioned above in naturally exposed animals.

3) A link between biotransformation and toxicity of a compound in standard toxicity assays, such as the microtox® and the mutatox® microbial assays.

4) Testosterone hydroxylase assay: The male steroid hormone testosterone can be hydroxylated at several positions by the cytochrome P450 system. In rats, each hydroxylation position has been assigned to a specific isoform. Thus, the metabolites formed allow an assessment of the active isoforms.

5) Seal cell lines will be established to study steroid binding capacity, and hormone transformation and elimination rates.

5.7 Vital Population Parameters and Study Areas

The major purpose of the ecological core programme is to develop models on population dynamics and energetics which would provide a linkage to studies of physiology and environmental contaminants. High quality data on population parameters, spatial structure and diet will be extracted from studies in areas where detailed background information is available.

The criteria used for the choice of selected study areas were that they describe a gradient in contaminant exposure, and that background data are available, or easily can be made available, for studies on population parameters. The most important background information on population parameters is population growth rate, age-specific mortality rate and fecundity. Data on at least two of these three variables should be available. An overview of available population parameters in the chosen groups is given in Table 6. For some populations, there are monitoring programmes on population dynamics or pollution status. The present project will be coordinated with those programmes.

Table 6. Populations with relevant background data and where live capture for blood sampling was considered feasible. CF-Condition Factor; GR-Growth Rate; PP-Pup Production; PR-Pregnancy Rate; ASM-Age at Sexual Maturity; D-Diet; B-Behaviour. Y-Information available; N-No information available; ?-Some information may be available.

Species	Population/ Area	CF	GR	PP	PR	ASM	D	B
Ringed Seal	Svalbard	Y	?	?	?	?	Y	Y
	Baltic Sea	Y	Y	Y	Y	Y	?	Y
Grey Seal	Liverpool Bay, UK[1]	N	Y?	Y?	Y?	Y	Y	?
	Froan, Norway	?	Y	Y	?	Y	?	Y
	Breidafjordur, Iceland	?	Y	Y	Y	Y	Y	?
	Baltic Sea	N	Y	Y	Y	Y	N	?
Harbour Seal	Wadden Sea	Y	Y	Y	Y	Y	Y	Y
	Skagerrak	Y	Y	Y	Y	Y	Y	Y
	West coast Norway	?	Y	Y	Y	Y	Y	Y
	Moray Firth, UK	?	Y	Y	Y	Y	Y	Y

[1]This area is not a breeding site but is a haul-out site favoured by grey seals outside the breeding season. Data on the movement of animals, obtained using satellite relay data loggers, will determine which is the relevant UK breeding population for this group of animals.

In all the populations listed, it is possible to obtain non-destructive sampling from neonates. The possibilities of capturing free-ranging juveniles and adults vary between the different populations. However, in most populations it is possible to obtain non-destructive sampling from at least 10 individuals of each subgroup (juveniles, adult males, and adult females, Table 6). Where available, by-catches and strandings from these species will be used for pathological studies.

A limited number of population parameters, which will give information on population dynamics and population energetics, should be studied in all populations during the project period. More intensive studies will be carried out on four specific populations of harbour seals along a pollution gradient in which population and behavioural data already exist, in the Moray Firth, Wadden Sea, Skagerrak and the west coast of Norway. Studies in these populations should be conducted using non-destructive methods and behavioural studies and by various tagging methods. It is also possible to catch animals from these four populations for controlled studies in semi-natural conditions. Facilities for keeping live animals exist in three of the four locations.

5.8 Participating Institutes

Tables 7, 8, 9, and 10, below, show institutes proposed by WGMMHA as potential participants in the programme. To some extent, the institutes listed below reflect the participation at the WGMMHA meeting. However, not all of these institutes were contacted prior to the meeting and the tables should therefore be taken as indicators of the types of institutes that may be involved. Additional institutes may also be involved, and North American research institutions are invited to participate. The University of California at Davis was mentioned as an institution that preferably may be involved because of their particular expertise in marine mammal immunology. Table 7 shows lead institutes for the analysis of each package of contaminants, and there should be close collaboration between these and other institutes for the exchange of reagents and samples (particularly for calibration exercises where the same assays are being carried out in different institutes). Table 8 shows possible lead institutes for indicator studies.

Table 7. Institutes which have been identified at the time of the WGMMHA meeting as possible participants for analysing the contaminant packages in the programme.

Package	Harbour seal plus prey species	Grey seal plus prey species	Ringed seal plus prey species
Core Programme			
1	IBN-DLO, Texel, Netherlands	CEFAS, Burnham-on-Crouch, UK	Norwegian National Veterinary Institute, Oslo, Norway
2	NIOZ, Texel, Netherlands	CEFAS, Burnham-on-Crouch, UK	[1]University of Stockholm, Stockholm, Sweden
Additional Programme			
3	Institute for Marine Sciences, University of Kiel, Germany	Institute for Marine Sciences, University of Kiel, Germany	Institute for Marine Sciences, University of Kiel, Germany
4	IVM, Free University, Amsterdam, Netherlands	IVM, Free University, Amsterdam, Netherlands	IVM, Free University, Amsterdam, Netherlands
5	Marine Laboratory, Aberdeen, Scotland	Marine Laboratory, Aberdeen, Scotland	Marine Laboratory, Aberdeen, Scotland
6	RIVO-DLO, Netherlands	Norwegian National Veterinary Institute, Oslo, Norway	Norwegian National Veterinary Institute, Oslo, Norway
7	[2]University of Stockholm, Stockholm, Sweden	[2]University of Stockholm, Stockholm, Sweden	[2]University of Stockholm, Stockholm, Sweden
8	[2]University of Stockholm, Stockholm, Sweden	[2]University of Stockholm, Stockholm, Sweden	[2]University of Stockholm, Stockholm, Sweden
9	Norwegian University of Science and Technology, Trondheim, Norway	Norwegian University of Science and Technology, Trondheim, Norway	Norwegian University of Science and Technology, Trondheim, Norway

[1]University of Stockholm, Bergman and Klasson-Wehler Lab.
[2]University of Stockholm, Jansson Lab.

Table 8. Indicator studies and participating institutes.

	Harbour seals	Grey seals	Ringed seals
Reproductive effects	IBN-DLO	NTNU	NTNU
Immune system effects	SMRU	NVH	NVH
Biotransforming enzymes	NIOZ	NVH	NVH
Other systems	IfM, University of Kiel	IfM, University of Kiel	IfM, University of Kiel

NVH-Norwegian College of Veterinary Medicine; NTNU-Norwegian University of Science and Technology; IBN-DLO-Institute for Forestry and Nature Research

Table 9. Captive studies and participating institutes.

	Core Programme	Additional Programme
	Harbour seals	Grey seals
Reproductive system	IBN-DLO	SMRU
Immune system	IBN-DLO	SMRU

SMRV-Sea Mammal Research Unit, UK

Table 10. Field programmes and participating institutes.

Core Programme		Additional Programme			
Harbour seal		Grey seal		Ringed seal	
Moray Firth, UK	SMRU	Liverpool Bay, UK	SMRU	Svalbard	Norwegian Polar Institute
Wadden Sea	IBN-DLO*	Froan, Norway	NTNU	Baltic	Swedish Natural History Museum
Skagerrak, Sweden	Swedish Natural History Museum	Breidafjordur, Iceland	MRI		
West coast of Norway	IMR Norway	Baltic	Swedish Natural History Museum		

*This institute will coordinate the research in the Wadden Sea.

5.9 Estimated Costs

To be completed after consultation with the nominated institutes.

5.10 Furthering the Project Proposal and Preparing Applications for Funding

The project proposal as agreed by WGMMHA will serve as the basis for developing applications for project funding. The Working Group nominated A. Bjørge, A. Hall, and P.J.H. Reijnders to act as an editorial group in furthering the project proposal with the aim of developing an application for submission to the EU Fifth Framework. P. Reijnders agreed to serve as coordinator for a possible EU-funded part of the project and a full time secretariat/administrative assistant for the project will eventually be employed at the IBN-DLO. A budget for entering relevant environmental data onto the ICES databases should be included in this programme.

Due to the comprehensiveness of the research plans and the costs involved, more than one source of funding should be approached. WGMMHA will in collaboration with interested institutes seek financial support from different sources, including national research councils and relevant management authorities within ICES Member Countries. North American research institutions are invited to participate in the programme.

6 METHODS AND NEW TECHNIQUES IN LIFE HISTORY STUDIES OF MARINE MAMMALS

The influence from skewed samples on estimates of population growth rate, age-specific mortality and fecundity can be substantial in populations with age- and sex-specific features. By quantifying the behavioural differences among age and sex classes, data from skewed samples can be compensated retrospectively. Awareness of the existence and the potential magnitude of such biases is highly relevant for the designs of surveys, sampling programmes and the implementation of management plans of age-structured populations (WP10).

The age- and sex-specific behaviour of harbour and grey seals can be studied by using freeze-branded animals. Because the brand is permanent and visible up to a distance of 500 m, the harassment is limited to one occasion in the lifetime of the seal (the catching day). The composition of harbour seal groups on land exhibit a conspicuous seasonal flux, and the fraction on land was not representative of the entire population at any time during the summer. The results have far-reaching implications since most studies of seals are carried out at haul-out sites, and differential behaviour between the sexes and among age classes is expected in all populations and species of seals. Skewed samples generate biases in estimates of population growth rate, age-specific mortality and fecundity (WP10).

Age-specific haul-out patterns must be taken into account when analysing data from populations with non-stable age structures. Surveys of the Swedish-Danish harbour seal population, which had distorted and changing age distributions after the 1988 seal epizootic, underestimated the population size by 6 % in 1988 and overestimated the same parameter by 13 % and 16 % in 1989 and 1990, respectively (WP10).

Tooth ultra-structure in the harbour porpoise was examined as a new method for differentiating between animals from different geographical regions of the North Atlantic. The teeth are useful indicators of differences because although their internal characteristics may be modified throughout time as they grow, they remain essentially unchanged once formed, and provide a permanent phenotypic record for the individual. Genetic information tells about historic origins, the breeding habits and longer-term affiliations. However, this information, used alone, may not be adequate in a practical way for present management purposes. Therefore, information based on genetics, morphology, and ecology (see Table 11) are desirable to make the best judgement about population discreteness (WP18).

WGMMHA agreed that this promising new technique might be applied to a variety of species, including seals, if modified. Combined with other investigations, this technique should provide valuable information toward the elucidation of population structure. It was noted that should the technique be applied to any other species, the appropriateness of characteristics and ultra-structure of the teeth must be carefully investigated prior to using them as discriminators for geographic groupings of individuals.

Table 11. Methods for testing hypotheses regarding putative sub-populations, and relative importance and limitations.

Method	H_0 False	H_1	H_2	H_3 False	Priority	Limitation
Tagging for movements	X	X	X		Medium/Low	Needs much effort; tells about individuals; does not tell about reproductive (genetic) mixing
Biological parameters					Medium/High	Generally stable information, but may be affected by the environment: food supply; pollutants; exploitation that can lead to trends over time; seasonal variation; tells about populations; can be labour intensive.
Time of breeding		X	X	X		
Life history parameters		X	X	X		
Parasite load		X	X	X		
Tooth ultrastructure		X	X			
Chemical Signals					Medium	Can provide clear-cut definitions of populations, but may change over time—years in pollutants, but maybe only months in fatty acid signatures; does not tell about reproduction; labour intensive
Organochlorine load		X	X			
Heavy metal load		X	X			
Isotope load		X	X			
Fatty acid signatures		X	X			
Morphology					Medium/High	Persistent in individuals; reflects genotype but may be affected by environment and ecology; tells about individuals and populations
Non-metric skull chars.	X	X		X		
Morphometrics of skeleton	X	X		X		
Tooth ultrastructure	X	X		X		
Genetics					High	Persistent; tells about individuals and populations; mtDNA is sex-linked; microsats focus on nuclear DNA; allozymes are phenotypic expression of genotype; all methods together are very powerful tools
mtDNA sequencing	X	X	X?			
DNA microsatellites	X	X	X?	X		
mtDNA RFLP	X?	?	?	X		
Allozyme electrophoresis	X	X	X?	X		

Key

H_0: 14 discrete populations; H_1: some number of populations (14 or less) with small amounts of mixing among them

H_2: some number of populations with large amounts of mixing between them; H_3: complete panmixia across the entire North Atlantic

WGMMHA agreed that this study demonstrated the importance of historical records when considering basic biological information. Such information can be used to investigate long-term trends and/or seasonal variation in a suite of biological parameters. However, it should be kept in mind that there are many factors that could introduce bias into some types of analyses of such data.

A review of published and unpublished historical records relating to distribution and abundance of harbour porpoises in Danish waters was presented from WP19. This information drew from directed catch data, and by-catch and strandings data. Biological information on diet, parasites, pollutants, biological parameters (age and reproduction), and body condition was reviewed. The review incorporated information from 1996–1998 as well as a comprehensive database containing 1,900 records from 1834 to the present (WP19).

7 HABITAT-RELATED TRANSMISSION OF PARASITES IN MARINE MAMMALS

7.1 Case Studies

WP7 provided information on harbour seal host population size versus abundance of the parastic nematode sealworm *Pseudoterranova decipiens*. In the central part of the Koster archipelago in the northern Skagerrak (Sweden), the population of harbour seals increased from 350 to more than 1000 indiviudals between 1988 and 1998. During the same period, the abundance of sealworms in highly infected cottid fish species did not show a corresponding increase. Because harbour seals do not feed on cottids, an infection route via cottids and their predator, Atlantic cod (*Gadus morhua*), was proposed.

WP19 reported identification, incidence and degree of infestation of parasites in ear/sinus, lung, heart, stomach, intestines, liver and kidney in harbour porpoises in Danish North Sea and inner Danish waters, in four time periods, 1943–1944, 1962–1965, 1985–1990 and 1996–1998. Infestation was consistently high in both areas and all time periods in ear/sinus, lungs and liver, although there appeared to be a decline over time in parasitic load in liver. Factors such as the age of individual host animals and time period should be considered in studies of habitat-related transmission of parasites.

7.2 Population-Level Effects of Parasites in Marine Mammals

In its discussion of furthering the work of WGMMHA on parasites in marine mammals, WGMMHA approached this subject from the perspective of population biology, and reviewed available evidence for both host- and parasite-mediated regulations of involved populations. This discussion was primarily based on a review provided in WP6.

7.2.1 Parasite-mediated regulation of host populations

Strong inference for regulation of vertebrate populations can be obtained by monitoring natural and human-induced introductions or removals of pathogens or by manipulation of host-parasite systems (Dobson and Hudson, 1995; Hudson and Dobson, 1995). Unfortunately, these approaches do not seem well-suited for the study of marine mammals. However, solid evidence for parasite-mediated regulation of marine mammal populations can still be obtained from field data if three conditions are fulfilled: (i) parasites must have an effect on host mortality or reproduction rates (the so-called lethal and sublethal effects, respectively), (ii) such effects must increase with host population size (that is, density-dependent), and (iii) the effects must be additive.

The latter is important because density dependency is a necessary but not sufficient condition to prove parasite-mediated regulation. In some cases, parasitism concurs with other factors, such as predation, hunting, shortage of resources, etc. Then, the impact of parasites might be compensatory, i.e., parasites eliminate or affect individuals which, in the absence of disease, would have been eliminated by the other factors. Regulation would occur only if the effect of parasites is additive, that is, 'in addition to' the other factors (Holmes, 1982; Scott and Dobson, 1989).

Evidence relevant to marine mammal populations is still fragmentary because no single study has addressed the three issues simultaneously, but the few investigations available can provide some ideas on methods for further work. Regarding the estimation of lethal and sublethal effects, practically all relevant studies have focused on the former (e.g., Perrin and Powers, 1980; Lambertsen, 1986). Sublethal effects are far more difficult to quantify for marine mammal populations although it has been proposed that certain parasites may affect the reproductive rates of odontocete populations (Geraci *et al.*, 1978; Raga and Balbuena, 1993).

Mortality rates can be estimated by combining information from pathological surveys, which provide information on the prevalence of disease conditions, together with epidemiological data. For instance, Perrin and Powers (1980) used age-stratified data to estimate the mortality caused by the nematode *Crassicauda* sp. in spotted dolphins *Stenella atenuatta* caught accidentally in fisheries in the Eastern Tropical Pacific. The parasite causes characteristic damage in the pterygoid bones. Assuming that the skull lesions are irreversible, the authors developed a model to describe variations in the prevalence of lesions with dolphin age. Such a model suggested that the death rate attributable to *Crassicauda* sp. was 11 % to 14 % of the natural mortality. However, sampling biases could affect this estimate if, for example, infected dolphins were more likely to be caught than uninfected ones. Therefore, the results of such studies must be interpreted with caution, because several alternatives should be evaluated to account for the patterns observed (see Lambertsen, 1986). Similar procedures can be used to study sublethal effects, based on techniques developed for other vertebrates (e.g., Szalai *et al.*, 1992).

Finding evidence for density-dependent constraints on host populations requires good historical estimates of both mortality or morbidity rates and population size. The best, and perhaps only, example of such studies in marine mammals concerns the mortality caused by the hookworm *Uncinaria lucasi* among pups of northern fur seals *Callorhinus ursinus* from the Pribilof Islands, Alaska. Based on surveys spanning about thirty years, Fowler (1990) showed that mortality rates increased with pup numbers, thus suggesting a density-dependent relationship.

The last question (whether parasite-induced effects are additive) can be difficult to answer, since one may need to know the host population size both with and without the parasite. Nevertheless, a good knowledge of death rates due to other factors can help to tackle the problem. Lambertsen (1986) studied the prevalence of lesions caused by *Crassicauda boopis* in the kidney, urinary tract and circulatory system of 87 North Atlantic fin whales *Balaenoptera physalus*. The lesions were highly prevalent, affecting about 95 % of the whales. Assuming a low virulence, the mortality rate attributable to *C. boopis* was estimated as 4.4–4.9 %, which represents a high proportion of the natural mortality rate of large whales (4–7 %). Since fin whales are large and fast-swimming, mortality due to predation and starvation do not seem large in comparison to that attributable to the parasite (Lambertsen, 1986). Thus, the data suggest that the effect of *C. boopis* on the population is truly additive and, hence, significant in terms of regulation.

The literature suggests that some host-parasite systems are more promising to study the issue of regulation of marine mammal populations. The above examples involved nematodes invariably. Particularly, species of *Crassicauda* deserve more attention since they live within the host tissue and seem highly destructive. In addition, these nematodes have not purposely been looked for in many parasitological surveys, because their detection is time-consuming as it involves extensive dissection. Therefore, their actual role in cetacean mortality might have been grossly overlooked. Other nematode-host associations amenable to such studies are *U. lucasi* from fur seals (see above), heartworms from seals (Measures *et al.*, 1997) and lungworms infecting seals and odontocetes (e.g., Onderka, 1989; Baker and Martin, 1992; Bergeron *et al.*, 1997). The role of morbilliviruses in regulating marine mammal populations can also be particularly rewarding. According to Harwood and Hall (1990), microparasitic, particularly viral, infections seem to be the most important factor influencing population size of marine mammals in the absence of human exploitation. Morvilliviruses seem to persist in some populations and can lead to occasional die-offs due to interspecific transmission to naive populations (de Swart *et al.*, 1995; Kennedy, 1998). The occurrence of periodic outbreaks might be related to host density. At least, the best documented case of morbillivirus epizootic, the Phocine Distemper Virus (PDV) die-off of the North Sea harbour seals in 1988, occurred after several years of drastic increases in the seal populations (Dietz *et al.*, 1989). However, accurate historical censuses should be available for other marine mammal populations before concluding a general density-dependent relationship. The issue of vertical transmission of morbilliviruses (Markusen, 1992; Visser *et al.*, 1993; Dye *et al.*, 1995) needs further work because this feature lowers the threshold host population required for parasite transmission, thus increasing the chances for host regulation (Anderson and May, 1979).

A final issue regarding viral epizootics of marine mammals is the influence of contaminants, particularly polychlorinated biphenyls, in the development of the outbreaks. Historical records suggest several viral die-offs of harbour seals long before the advent of anthropogenic pollutants (Harwood and Hall, 1990). However, the question whether these substances play a role in the incidence, severity or periodicity of viral epizootics is still open, having both strong advocates (de Swart *et al.*, 1995; Ross *et al.*, 1996) and detractors (Kennedy, 1998). Obviously, more work is needed to obtain a long- or medium-term database of contaminants present in animals affected by epizootics. Such information would allow the development of epidemiological models, which presumably would provide testable predictions. In addition, the North Sea seal population has been considered too small to host the morbillivirus. It is thought that a new viral outbreak could only arise through re-introduction from outside the area (Swinton *et al.*, 1998). One of the assumptions in this study was that the infection rate was kept constant. WGMMHA noted that if the virus has become epizootic, this assumption may be invalid.

7.2.2 Host-mediated regulation of parasite populations

The interactions between host and parasite populations are bilateral and, therefore, the host can also regulate parasite populations (Smith, 1994). The role of marine mammals in the transmission of parasites is particularly relevant when the economy or public health are at stake. Most studies have focused on anisakid nematodes and, particularly, on the sealworm *Pseudoterranova decipiens*.

Increases in sealworm abundance in fish fillets have been related to parallel increases in grey seals (Zwanenburg and Bowen, 1990; Burt, 1994). This suggests a density-dependent (regulatory) process and supports the view that culling of seals would limit infections in fishes. However, culling programmes are controversial because economic considerations are confronted with the ethics of environmental management. In addition, the relationship between seal and worm abundance is still poorly understood because the sealworm (like other anisakids) has a complex life cycle and the transmission may depend on many parameters.

Mathematical models can be used to investigate the relationship between seal abundance and parasite numbers in fish. For instance, des Clers (1990) and des Clers and Wootten (1990) studied the change in the basic reproductive rate of the parasite, R_0, with the number of seals and cod. (R_0 is an epidemiological parameter which measures the ability of the parasite to spread.) The models showed that, all other parameter values being fixed, an increase in the number of cod would have a greater influence in rising R_0 than a corresponding increase in the number of seals. This suggests that fish are a greater reservoir of parasites than seals and that control should concentrate primarily on the intermediate hosts rather than on the definitive ones.

In addition, seal culling might not reduce the parasite population to affordable levels. Support for this idea comes from a unique natural experiment propitiated by the 1988 PDV epizootic. des Clers and Andersen (1995) examined the effect of the seal die-off on infection levels in fish in Hvaler, Norway. The study showed that a reduction as drastic as two thirds of the seals led only to a one-half decrease in sealworm mean abundance.

Selective culling has been contemplated as an alternative to random culling of seals (Stobo *et al.*, 1990), which requires additional work to identify the target individuals. However, such programmes should be designed with caution because selective culling can change the genetic makeup of the population in few generations (Hartl *et al.*, 1995), with unpredictable long-term effects.

Clearly, more work with mathematical models is needed before they can be used as decision-making tools. Additional field studies attempting to characterise the infections in different intermediate and definitive hosts (e.g., Brattey and Bishop, 1992; Aspholm *et al.*, 1995; Boily and Marcogliese, 1995; Marcogliese, 1996, 1997) are needed (see below). Such investigations allow the inference and estimation of worm population parameters such as the abundance, prevalence, dispersion indices, fecundity, etc., which can be incorporated into mathematical models (see a simple example below). Recent studies have provided information on sealworm transmission from invertebrates to fish (Marcogliese, 1996) and the role of non-commercial fish in the life cycle (Aspholm *et al.*, 1995; Jensen and Andersen, 1995). Some data can be crucial for further modelling. For instance, Marcogliese (1997) showed a lack of density-dependent effects within the sealworm populations, whereas the models of des Clers (1990) and des Clers and Wooten (1990) assumed that the sealworm populations were regulated intrinsically in a density-dependent manner.

An interesting topic that has received little attention, is the role of sympatric marine mammal species in the transmission of anisakids. Ecologists long have recognized that all species capable of hosting a parasite species do not play equivalent roles to maintain the parasite population. Logically, defining the host populations which contribute most to the maintenance of the parasite population is of paramount importance for designing control strategies.

Traditionally, the prevalence (percentage of hosts infected), the intensity (average number of parasite individuals per infected host) and/or the reproductive potential of parasites (usually measured as the relative proportion of mature worms, more rarely, as estimates of fecundity) have been used to identify primary, secondary, and unsuitable hosts for any particular parasite species. A recent example, involving marine mammals and anisakids, has been provided by Kuramochi *et al.* (1996). These authors analysed the parasites of the stomach of 21 minke whales *Balaenoptera acutorostrata*, 87 Dall's porpoises *Phocoenoides dalli*, 17 Pacific white-sided dolphins *Lagenorhynchus obliquidens*, and 6 northern right whale dolphins *Lissodelphis borealis* in the Northwestern North Pacific. Differences in prevalence, intensity, proportion of adult worms, and body size of *Anisakis simplex* among these hosts led the authors to suggest that minke whales are the most important final hosts in the study area, while the smaller odontocetes appeared to play a relatively minor role in the life cycle.

However, the measurement of these variables is a necessary, but not sufficient, condition to identify the potential for transmission by each host within a host community (Holmes *et al.*, 1977). Actually, the key question is *the relative rate of flow* of parasites through each host population. Apart from the variables mentioned above, this involves consideration of the relative sizes of the host population in the local community, as well as the turnover of parasites in them (see Brattey and Stenson (1993) for an example of anisakids in seals). To illustrate this, Holmes *et al.* (1977) used the example of *Schistosoma japonicum* in the Philippines. Schistosomes have a very high prevalence, and females had their largest life span and total egg output, in humans. However, the *S. japonicum* population might rather be maintained by field rats, due to their large host population (far larger than that of humans) and the higher probability of contact with the parasite through snail consumption.

The relative flow for each host (*RF*) can be calculated as $A \times P \times W$, where A is the mean abundance of the parasite (prevalence times mean intensity); P is the relative size of each host population and W is a coefficient to adjust to a proportion of the total flow to all host species. The relative output of eggs (*OE*) from each host species can be calculated as $A \times F \times P \times W'$, where F is the proportion of gravid females in that host and W' is a coefficient to adjust to a proportion of the total egg output to all host species. The ratio *OE/RF* allows identification, *prima facie*, of the role of each host in maintaining the parasite population. It is interesting to note that species having very low ratios can act as an ecological 'sink' for the system because they recruit parasites but contribute little or nothing to producing new infective stages.

Although the above model seems fairly crude, WGMMHA considered that its rationale is useful, providing that future refinements will make the approach more realistic. WGMMHA briefly discussed some preliminary problems regarding the model and its goals as applied to anisakids infecting marine mammals:

1) *A* must be accurately estimated. Anisakids typically exhibit aggregate distributions in their definitive marine mammal hosts (see, e.g., Stobo *et al.*, 1990; Brattey and Ni, 1992; Brattey and Stenson, 1993; Marcogliese *et al.*, 1996). In this context, reliable estimates of the mean abundance depend heavily upon host sample size and the degree of parasite aggregation (Gregory and Woolhouse, 1993). Researchers should deal carefully with this problem before comparing and interpreting sample estimates of parasite mean abundance in several host species.

2) *F* is not an accurate measure of the relative reproductive success. Instead, it is a rather crude estimate of this parameter. At one extreme, the recurrent occurrence of non-gravid females in a host species, regardless of parasite population size, rules out any role of this host for transmission. However, what happens when *F* values are alike? Clearly, we need to improve our understanding of how host effects influence the parasite reproductive output. For instance, as a measure of fecundity in *P. decipiens*, Marcogliese (1997) determined uterine egg counts by means of an electronic particle counter. If the influence of confounding variables is minimised (see Marcogliese, 1997, and references therein), this procedure can add fundamental information for inter-host comparisons. Other variables, e.g., worm size, could be potentially used as an additional information source on reproductive success (e.g., Kuramochi *et al.*, 1996).

3) The boundaries of the local host community are blurred. In contrast with largely closed systems, such as ponds or lakes, in the coastal or pelagic realm it is much more difficult, or impossible, to identify the spatial scale upon which a local host community should be defined. It seems clear that, at a large geographical scale, the same species may play opposite roles for nematode transmission to local fish stocks. For instance, the general rarity of *A. simplex* in harbour porpoises *Phocoena phocoena* off eastern Canada contrasts with the much higher abundance of *A. simplex* in harbour porpoises from British waters (Brattey and Stenson, 1995). In some cases, it might be possible to define operational boundaries for local host communities (at least temporally), based on a good knowledge of the area and the ecology of the potential host species involved in the cycle. What is peremptory, however, is to learn more about habitat-related processes and processes of spatial dispersion of anisakid larvae.

7.3 Recommendations Regarding Habitat-Related Transmission of Parasites

WGMMHA pointed out the need for long-term investigations. Data on host population sizes, pathology and parasitological discriptors should be gathered during several years because of the time scale of the processes involved. The spatial distribution of hosts should be monitored to understand the habitat-related transmission processes, and the areas and exposures which the mobile hosts are integrating. Differences in host parasite communities may also contribute to understanding population structure of marine mammal host species.

WGMMHA recommended that ICES Member Countries facilitate research along these lines. WGMMHA will explore the possibilities for international funding for future collaborative research programmes in this field, and WGMMHA will return to this subject within the next three years.

8 REVIEW OF NEW INFORMATION ON HEALTH STATUS AND EFFECTS OF CONTAMINANTS ON MARINE MAMMALS IN THE BALTIC SEA

HELCOM has requested ICES to continuously assess the condition of the harbour porpoise and the seal populations of the Baltic Sea on the basis of new evidence presented by the Contracting Parties and other relevant information. HELCOM has requested that an evaluation of the populations of these species be conducted every third year. This evaluation would include population sizes, distributions, migration patterns, reproductive capacity, effects of contaminants and health status, and additional mortality owing to interactions with commercial fisheries.

The next year in which a full evaluation would be conducted is in the year 2000. At the present meeting, WGMMHA had available new information on seasonal activity patterns and area use of ringed seals (WP9), development of Baltic grey and ringed seal populations during the 20th century (WP16) and a discussion of ringed seal population collapse correlated to organochlorine exposure (WP17).

In WP16, the reconstructions of the developments of the Baltic ringed and grey seal populations during the past century were described. These reconstructions were based on uniquely detailed hunting records from the Nordic countries and published data on demographic parameters. A projection model was elaborated where annual variations in the composition of catches by sex and age were taken into account. Grey seals decreased from 88,000–100,000 in the beginning of the century to approximately 4,000 in the late 1970s. The Baltic ringed seal population decreased during the same period from 190,000–220,000 to approximately 5,000. In the mid-1960s, the remaining populations were afflicted by sterility, probably caused by organochlorines, which inhibited natural growth during 25 years. Thus, the decrease in seal numbers was a consequence of excessive hunting, but the low numbers at present are due to lowered fertility rates after 1965.

In WP17 it was noted that concentrations of organochlorines in extractable fat from Baltic seals increased ten-fold during the latter half of the 1960s, a time after which a significant increase was detected in the prevalence of pathological changes of reproductive organs, skeleton, integument and intestines. Data on the present growth rate of the population (5 %) and pregnancy rates were used to model the flux in reproductive capacity in the past. It was found that irrespective of which age structure is used in the model, the growth rate was negative in the period 1970–1984. Including detailed information on hunting mortality shows that the population in the Bothnian Bay decreased from about 14,000 to less than 4,000 during the period 1960 to 1986. Only after 1998 was a positive trend possible. Significant correlation is found between the reported reproductive capacity of ringed seals and the concentrations of PCBs in extractable fat from seals. The flux in reproductive capacity is also significantly correlated to changes in concentrations of PCBs in the major prey item of ringed seals, the Baltic herring.

WGMMHA thanked T. Härkönen for his contribution of new information on Baltic Sea seal populations, and recommended that the discussion of population development in relation to OCs be expanded to include grey seals prior to next year's meeting of WGMMHA.

WGMMHA underlined the importance of the submission of information from all range states of the Baltic Sea for a comprehensive review of the status of marine mammal populations in the Baltic Sea, as requested by HELCOM.

For this next triennial review, WGMMHA suggested joint sessions with the WGMMPD to assess the status of Baltic marine mammal populations from both a qualitative (on the level of individuals) and quantitative (on the level of populations) approach.

9 CONTRIBUTIONS TO THE STRATEGIC PLANNING OF THE ICES MARINE HABITAT COMMITTEE

WGMMHA decided to postpone this discussion until further instructions and information on the Council's and Committee's strategic objectives become available.

10 FUTURE ACTIVITIES OF THE WORKING GROUP ON MARINE MAMMAL HABITATS

WGMMHA will pursue its future work in accordance with the activities identified in the 1998 WGMMHA report (ICES CM 1998/E:6). The WG may at future meetings adjust these activities to implement the outcome of the ICES strategic planning process (cf. Item 9, above). In light of the achievements made by WGMMHA this year, the WG will for its year 2000 meeting give priority to:

1) studies of marine mammal habitat requirements including spatial and temporal aspects of habitat use and foraging ecology and other topics of relevance for marine mammal exposure to contaminants. The WG will give priority to this topic with the objective of within a two-year time frame developing a proposal for an international collaborative research programme.

2) studies aimed at improving methods and techniques for improved precision in estimation of life history parameters. The WG will identify projects and explore possibilities for their funding.

3) review progress in the research on cause-effect relationships of contaminants in pinnipeds, as planned at the 1999 meeting of WGMMHA.

11 RECOMMENDATIONS

The Working Group on Marine Mammal Habitats [WGMMHA] (Chair: Dr A. Bjørge, Norway) will meet in 2000 at a venue and date to be decided to:

a) review progress in studies of marine mammal habitat requirements, including spatial and temporal aspects of habitat use, with emphasis on topics of relevance for marine mammal exposure to contaminants;

b) evaluate, in cooperation with the WGMMPD, the populations of grey seals (*Halichoerus grypus*), harbour seals (*Phoca vitulina*) and ringed seals (*Phoca hispida botnica*) and harbour porpoises (*Phocoena phocoena*) in the Baltic Sea, including distribution and migration, effects of contaminants, health status and reproductive capacity;

c) review invited papers and other available documents on the status, recent achievements and new ideas for progress in techniques and methodology for life history studies, including defining specific projects and evaluating and exploring possibilities for their funding;

d) review progress in the research on cause-effect relationships of contaminants in pinnipeds, as planned at the 1999 meeting of WGMMHA.

WGMMHA will seek joint sessions with the Working Group on Marine Mammal Population Dynamics and Trophic Interactions (WGMMPD) to conduct a comprehensive review of the status of Baltic marine mammal populations. The Chair of WGMMHA will liase with the Chair of WGMMPD to explore possibilities for convening the Working Groups at times and venue that facilitate optimal participation of the groups.

Justifications:

Items a, c, and d are justified by the ICES Five-Year Science Plan.

Item b is justified by the request from HELCOM.

The recommendations are also found in Annex 4.

12 OTHER BUSINESS

No other business was raised.

13 ADOPTION OF THE REPORT

The report was adopted, as amended, by the Working Group on 12 March 1999.

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ANNEX 2: AGENDA

1 Introduction and meeting arrangements

2 Appointment of rapporteurs

3 Terms of reference and agenda
- 3.1 Terms of reference
- 3.2 Adoption of agenda

4 Review of progress in studies of marine mammal habitat requirements
- 4.1 Habitat use
- 4.2 Overview of methods for studies of habitat use based on identifying and tracking individuals in a population
- 4.3 Ethics and effects of study techniques on animals
- 4.4 Recommendations

5 Proposal for research on cause-effect relationships between contaminants and population-level effects in pinnipeds
- 5.1 Introduction
- 5.2 Objectives
- 5.3 Compounds of relevance to toxic effects in seals
- 5.4 Responses and indicators of toxicity (Biomarkers)
- 5.5 Confounding factors
- 5.6 Laboratory experiments
- 5.7 Vital population parameters and study areas
- 5.8 Participating institutes
- 5.9 Estimates of costs
- 5.10 Furthering the project proposal and preparing applications for funding

6 Methods and new techniques in life history studies of marine mammals

7 Habitat-related transmission of parasites in marine mammals
- 7.1 Case studies reported to the Working Group
- 7.2 Approaches to study population level effects of parasites in marine mammals
- 7.3 Working Group recommendations regarding habitat related transmission of parasites

8 Review of new information on health status and effects of contaminants on marine mammals in the Baltic Sea

9 Contributions to the strategic planning of the ICES Marine Habitat Committee

10 Future activities of the Working Group on Marine Mammal Habitats

11 Recommendations

12 Other business

13 Adoption of the report

ANNEX 3: LIST OF WORKING PAPERS

WP1 Siebert, U., and Wünschmann, A. 1999. Pathological investigations on marine mammals for pollutant-effect monitoring.

WP2 Ruus, A., and Skaare, J.U. 1999. Suggestions for future research on responses to environmental contaminants in marine mammals.

WP3 Hall, A.J. 1999. The effects of contaminants on marine mammal populations: Considerations in the design of a research project.

WP4 Lunneryd, S.G. 1999. Food preference of harbour seals (*Phoca vitulina*) in baited cages.

WP5 Reijnders, P.H.J. 1999. Reproductive and developmental effects of endocrine disrupting chemicals on marine mammals.

WP6 Balbuean, J.A., Aznar, F.J., Herreras, M.V., Fernández, M., and Raga, R.A. 1999. Parasite transmission and marine mammals: a research programme in population ecology.

WP7 Lunneryd, S.G., Ugland, K.I., and Aspholm, P.E. 1999. Increasing population size of harbour seal (*Phoca vitulina*) in the Skagerrak does not influence the infection rate of sealworms (*Pseudoterranova decipiens*) in the benthic cottid *Taurulus bubalis*.

WP8 Hårding, K., and Härkönen, T. 1999. Site fidelity, and mechanisms causing spatially structured populations of harbour seals (*Phoca vitulina*).

WP9 Härkönen, T., Jüssi, M., Hårding, K., Jüssi, I., and Helle, E. 1999. Seasonal activity patterns and area use of Baltic ringed seals (*Phoca hispida botnica*).

WP10 Härkönen, T., Hårding, K., and Lunneryd, S.G. 1999. Age and sex specific behaviour in harbour seals (*Phoca vitulina*) leads to biased estimates of vital population parameters.

WP11 Beineke, A., Siebert, U., Wünschmann, A., Stott, J.L., and Baumgärtner, W. 1999. Investigations on the cross reactivity of selected leukocyte-marketers from various species for the characterization of lymphoid cells in harbour porpoises (*Phocoena phocoena*).

WP12 Stott, J.L., Funke, C., King, D.P. Blanchard, M., and Siebert, U. 1999. Development and application of reagents and techniques for assessing the immunologic health of marine mammals.

WP13 None.

WP14 Tolley, K.A. 1999. Radionuclide levels (137Cs) in harbour porpoises (*Phocoena phocoena*) from the Barents Sea and North Sea.

WP15 Härkönen, T., and Heide-Jørgensen, M.P. 1999. Recovery of seal stocks in the Kattegat-Skagerrak and the Limfjord after the seal epizootic.

WP16 Hårding, K., Härkönen, T. 1999. Developments of the Baltic grey seal (*Halichoerus grypus*) and ringed seal (*Phoca hispida*) populations during the 20th century.

WP17 Härkönen, T., Olsson, M., and Hårding, K. 1999. Ringed seal collapse correlated to OCs.

WP18 Lockyer, C. 1999. Application of a new method of investigating population structure of harbour porpoise, *Phocoena phocoena*, with special reference to the North and Baltic Seas.

WP19 Lockyer, C., and Kinze, C. 1999. Status and life history of harbour porpoise, *Phocoena phocoena*, in Danish waters.

ANNEX 4: RECOMMENDATIONS

The Working Group on Marine Mammal Habitats [WGMMHA] (Chair: Dr A. Bjørge, Norway) will meet in 2000 at a venue and date yet to be decided to:

a) review progress in studies of marine mammal habitat requirements, including spatial and temporal aspects of habitat use, with emphasis on topics of relevance for marine mammal exposure to contaminants;

b) evaluate, in cooperation with the WGMMPD, the populations of grey seals (*Halichoerus grypus*), harbour seals (*Phoca vitulina*) and ringed seals (*Phoca hispida botnica*) seals and harbour porpoises (*Phocoena phocoena*) in the Baltic Sea, including distribution and migration, effects of contaminants, health status and reproductive capacity;

c) review invited papers and other available documents on status, recent achievements and new ideas for progress in techniques and methodology for life history studies, including defining specific projects and evaluating and exploring possibilities for their funding;

d) review progress in the research on cause-effect relationships of contaminants in pinnipeds, as planned at the 1999 meeting of WGMMHA.

WGMMHA will seek joint sessions with the Working Group on Marine Mammal Population Dynamics and Trophic Interactions (WGMMPD) to conduct a comprehensive review of the status of Baltic marine mammal populations. The Chair of WGMMHA will liase with the Chair of WGMMPD to explore possibilities for convening the Working Groups at times and a venue that facilitate optimal participation of the groups.

Justifications:

Items a, c, and d are justified by the ICES Five-Year Science Plan.

Item b is justified by the request from HELCOM.