Guideline for monitoring reproductive status of seals in the HELCOM area

1. Background

The aim of this document is to define common practices to monitor the indicator ''Reproductive status in marine mammals'', one of the CORESET indicators agreed upon by HELCOM to assess environmental status within HOLAS II. Functional indicators require continued and synchronized monitoring among countries in the region. The indicator ''Reproductive status in marine mammals'' is part of the HELCOM program ''Health status'' in the program ''Mammals''.

Reproductive status of seals is an indicator of high relevance for the society, management and nature conservation for two main reasons: 1. Changes in reproduction success have direct influence on population growth rate and hence abundances of marine mammal populations 2. Pathological changes of the reproduction may be caused by anthropogenic activities such as hormone disruptive chemicals. Thus, declines in reproductive rates should be an important part of population monitoring programs and may warrant action by the society (such as hunting regulations and further search for harmful xenobiotics) to prevent un-controlled population declines and adverse human health effects.

There are three regularly occurring seal species in the HELCOM area (i.e. the Baltic Sea and Kattegat), harbour seal (*Phoca vitulina*), ringed seal (*Pusa hispida*) and grey seal (*Halichoerus grypus*). All three have different distribution and migration patterns, different habitat use and different feeding habits, and are consequently exposed to partly different environmental stressors. Also the reproductive biology differs among the marine mammals species of the HELCOM area. However, necropsies and assessement of reproduction biology parameters should be conducted based on similar protocols and are performed in exactly the same way for all three species. In the current document the methodology for monitoring reproductive status by necropsies of seals is summarized.

1.1 Introduction

Baltic seals live in one of the sea regions of the world with the highest anthropogenic activities. E.g. bioaccumulation through the food chain especially fat solvable chemical substances reach high levels in Baltic pinnipeds. Some contaminants such as polychlorinated biphenyls (PCB) and other similar substances (POPs) are hormone disruptive and may cause pathological changes in the reproductive biology. A disease complex that may be related to POPs was first described in Baltic seals (Bergman and Olsson 1985). Fecundity can also drop temporarily in healthy females due to natural causes, such as population density (populations approaching carrying capacity).

Aspects of reproduction relevant to monitoring

1. Pregnancy and birth rates

Pregnancy rate is here defined as the proportion of sexually mature females that bear a fetus/embryo in a given year in a post implantation sample. It can be estimated by inspection of reproductive organs from dead seals after the period of implantation. Sexually mature females can be distinguished by that they have ovulated, which can be seen in their ovaries, where a *corpus luteum* has been formed. It is known that average age at sexual maturity can change among years (Harding and Härkönen 1995), often a result from variations in food supply. Birth rate is defined as the proportion of mature females with *corpus albicans*/placental scars in a pre-implantation sample. Also pregnancy rate in healthy seals can vary because of natural causes (for example due to food shortage causing mal nutrition). The difference between birth rate and pregnancy rate indicates fetal mortality. A common practice is to calculate pregnancy rate from autumn samples and birth rate from spring samples.

2. Pregnancy/birth rate in seals with pathological changes
Pregnancy/birth rate can be reduced due to pathological changes in the uterus and the ovaries. Such changes may be part of a disease complex (the Baltic seal disease complex) that signals exposure to POPs. Some pathological changes also occur spontaneously at low frequency in otherwise healthy populations (for example tumors in older seals).

3. Pathological changes that do not affect fecundity
All pathological changes in the reproductive organs of females and males shall be registered also if they are not judged to affect fecundity or to belong to the Baltic seal disease complex. They may indicate changes in the environment or appearance of new environmental contaminants or infectious diseases and should be followed up.

4. The Baltic seal disease complex connected to PCB and DDT exposure
The pathological findings include inflammation on digits and claws, intestinal ulcers, vascular changes, present as arteriosclerosis, adrenal cortical hyperplasia, lesions in the kidneys, skull bone lesions and changes in the reproductive organs of females. One special finding in both ringed and grey seals is complete or partial occlusions of the uterine horns. Also uterine tumors (leiomyoma) were frequently found in older grey seal females. Substances like PCBs and DDTs are the most likely causes. Some disease processes are characterized by changes in the size of organs, and therefore weight of organs is included in the routine monitoring. Criteria for classification of different degrees of organ changes can be found in Bergman (1999) and Bergman and Olsson (1985). Full necropsies follow a standardized schedule that include almost 90 measurements points, and pathological changes are classified from 0 (normal) to 3 (severe injury) (Appendix 1)

Species included in the monitoring of reproductive status of seals

Ringed seal
The ringed seal is an ice breeding seal which depends on favourable ice conditions for successful reproduction (Sundqvist et al. 2012). Ringed seals prey on smaller fish, such as herring, sprat, and vendace, but also stickleback and crustaceans (Idotea). The natural variation in reproductive status depends on prey availability during the summer and autumn when blubber layers are built up. But breeding success is highly dependent on the availability of suitable ice cover. During years with little ice cover ringed seal pups have very high mortality rates.

Ringed seals also accumulate POPs to very high levels. Pregnancy rate dropped to 17% in ringed seals in the Bothnian Bay during the 1970s (Helle 1980), although no indices of starvation were seen in the seals. Recent findings indicate that the overall health problems due to environmental toxins have decreased during the past decades (Nyman *et al.* 2002, Routti 2009).

Grey seal
Grey seal reproduction is also favoured by good ice conditions since pup mortality is higher when females are forced to breed on land. Pup condition on ice is considerably better since they are 20% heavier at weaning as compared with pups born on land (Jüssi *et al*. 2008).

Grey seals prey on larger fish than ringed seals, and target e.g. herring, cod, flat fish and salmon. They have also suffered from sterility caused by occlusions of the uterine horns at high frequencies during the 1970s and onwards (Bergman *et al*. 1981; 2009). However, the reproductive health of grey seals has improved considerably over the past decades and is approaching normal levels (Bergman 2009, Bäcklin et al 2013).

Harbour seal
Harbour seals are not adapted to breed on ice and are confined to the southern and western parts of the Baltic Sea (Kalmarsund) and Kattegat. Levels of PCB has never reached as high levels as in the grey and ringed seals and there are no indications of that their reproduction is affected by these pollutants. However, levels of PCB found in harbour seals have been shown to affect their immune system functions

(Schwarz 19xx), and bone formation (Mortensen et al. 1992). Harbour seal fecundity is indicated to be close to its maximum at about 95% of adult females breeding annually during good years (with good prey availability) (Härkönen and Heide-Jørgensen 1990).

Different ecology
The three species mentioned above most likely also have differences in metabolism and physiology that may affect the rate of metabolising contaminants. The differences in ecology and possibly physiology indicate that trends in reproductive status (for example rates of occlusions of uterine horns) may have different underlying causes and may signal different stressors in the environment.

1.2 Purpose and aims

In short the monitoring of reproductive status is important to:

1. Understand population trends and abundances of Baltic seals

Seal populations may decline due to increased mortality or lowered fecundity. Monitoring reproductive status will make it easier to understand changes in population growth rate and thus help to discover causes behind a population decline, and guide conservation actions. Population trend and abundance is an indicator within the HELCOM CORESET program alongside with nutritional status, reproductive status and seal distribution.

The aim of the monitoring is to provide relevant data enabling assessment of the reproductive status of the seal species in different seal management units. Thus, the monitoring has a spatial component, where data can be used to assess spatial differences.

2. Obtain an early warning of new toxic substances in the environment and follow the development of known harmful substances

Reproductive status of seals has been proved to be a sensitive indicator of hormone disruptive substances (Bergman and Olsson 1985). Other fish eating mammals are likely to be exposed to the same substances but in lower concentrations. Effects of exposure have been shown also in top bird predators, otters and mink (Bignert et al. 1995).

2. Monitoring methods

2.1 Monitoring features

Many of the seals that are hunted are not sampled and neither are all by-caught and stranded seals. To increase the numbers of samples, samples requested from the current hunt are limited to e.g. lower jaw and reproductive tracts to facilitate the handling and to secure important information that would otherwise be lost. A smaller number of seals (depending on funding) are chosen for full necropsies where all entries in the protocol in Appendix 1 are examined.

2.2 Time and area

Spatial units shall strive to conform to the areas also used for analysis of the population trend estimates. However, in cases where sample sizes are limited, the data from several regions will be pooled for the statistical tests. In the Baltic HELCOM area, harbour seals are distributed in four units, which are monitored for population trends separately, namely the Limfjord, Kattegat, southwestern Baltic and Kalmar Sound. Ringed seals are distributed in four HELCOM units, which are monitored separately, namely the Bothnian Bay, the Archipelago Sea, the Gulf of Finland and the Gulf of Riga including Estonian coastal waters. Grey seals occur in all regions of the Baltic, but do not form a functional population in the Kattegat.

2.3 Monitoring procedure

2.3.1 Monitoring strategy

Data for the indicator are collected during seal necropsies by established veterinarians and specially educated biologists. The uterus is examined for fetus/embryo in post-implantation samples or postpartum signs (placental scars), and ovaries are examined for *corpora lutea* and *c. albicancia* in pre-implantation samples. Further investigations are performed by established macroscopic methods within pathology (Appendix 1) as well as other techniques such as microscopy (histology-, immune-histochemistry) as well as established techniques within parasitology, bacteriology and virology (See reference list for common practice of different techniques). Samples are collected year round.

To be measured:

- Pregnancy rate is measured as per cent sexually mature females (6-24 years old) having a fetus/embryo after the period of implantation, birth rate is measured as per cent of sexually mature females (7-25 years old) with postpartum signs (*corpus albicans* and/or placental scars) between the time of birth and implantation.
- Proportion of mature young females (2-5 years old) is measured as presence of *corpus luteum* in ovaries between the mating season and implantation.
- Proportion of females showing uterine pathological changes is examined year round.

Monitoring methodology

The monitoring methodology is described in detail in Bäcklin et al 2013.

2.3.2 Sampling method(s) and equipment

In Sweden, by-caught and stranded dead seals are sent to the museum of Natural History in Stockholm (NRM) where full necropsies are performed. A special phone number that also private persons can use 46-08-5195 51 44 makes it possible to quickly retrieve found dead seals. Similar facilities are found in Busum in Germany, the University of Aarhus in Denmark. LUKE, Finland, collects samples of hunted and by-caught seals (every year: lower jaw, reproductive organs and liver, and other organs, depending on the year). Subcutaneous blubber thickness and body length are measured by hunters/fishermen in the field, and the seals are weighed when possible. The samples are examined in cooperation with Evira (Finnish Food Safety Authority, Oulu).

Macroscopic inspection of reproductive organs can be used as a general assessment of reproductive status in the field if samples cannot be sent to a full screening. Selected specimens are transported to a laboratory for full necropsies. These labs are fully equipped for all pathological investigations.

2.3.3 Sample handling

The detailed dissection protocol for reproductive health is attached as Appendix 1. Most important information resulting from necropsies include presence of foetus/embryo, scar of placenta, weight of placenta, weight/length of ovaries, left and right ovaries separately, signs of *corpus luteum* and *albicans* in ovaries (sliced in 1-2 mm thick slices), signs of pathological changes, tumors and occlusions. Furthermore, malformations are noted and taken to further analysis. Depending on the cause of death and the time since death investigations of different detail can be performed. Plastic gloves are a mandatory in all handling of dead seals.

Histological preps are taken from specimens not frozen earlier and from pathological changes. Fixed and dyed preps are inspected in microscopes, and photographed with digital cameras. Catalogues are kept for both preps and photos. Samples used for chemical analyses are frozen at 20 °C, and stored at environmental tissue banks. Age determination is performed by staining histological sections of lower canine teeth and counting the annual growth layers (Lockyer et al 2011).

2.4 Data analysis

The optimal monitoring should encompass sufficient numbers of samples from all species of seals in all areas where they occur. The best scenario would be to collect and analyze all adult female seals, but due to financial constraints a limited number is processed annually. A theoretical number of about 30 adult females from each region annually would provide a good picture of the reproductive status in the area and allow detection of changes from year to year. However, for smaller samples, pooling the data of several years is required before sample size allows statistical testing and a more coarse but still valuable picture is obtained. Thus, in many cases the data need to be integrated over several years in order to reach sufficient statistical power. The number of years depends on sample sizes. Calculating e.g. three-year moving averages can be done when the sample size differs much between years to get a good picture of the reproductive success of seals.

Obtained data is tested against a set threshold value at 80% pregnancy/birth rate by a Bayesian analysis, where it is evaluated whether observed data support the determined threshold value of good status. In this process, 80% support for a pregnancy rate ≥ threshold value is required. If the unit fails to reach good status, the probability distribution is used to evaluate the confidence limits of the assessment. The package 'bayesm' in the program R has been used for the analysis.

Estimating age at sexual maturity at the population level is done by published standard equations, see for example Harding and Härkönen (1999).

3. Data reporting and storage

Format of reporting data is not yet agreed upon, but initiatives have been taken to compile national data annually by the HELCOM Seal Expert Group.

The data collected and currently used in the indicator are based on national data bases. Much of Swedish and Finnish data have been merged. German and Polish data remain to be included

Responsible organizations:

Swedish Museum of Natural History
Department of environmental research and monitoring
Box 50007
S-10405 Stockholm

Natural Resources Institute (LUKE)
Viikinkaari 4
FI-00790 HELSINKI
FINLAND

Aarhus University, Faculty of Science and Technology
Department of Bioscience, Frederiksborgvej 399
PO Box 358, DK-4000 Roskilde, Denmark

Institute for Terrestrial and Aquatic Wildlife Research (ITAW)
University of Veterinary Medicine Hannover, Foundation
Bischofsholer Damm 15, 30173 Hannover, Germany
Werftstr. 6, 25761 Büsum, Germany

Hel Marine Station
84-150 Hel
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4. Quality control

4.1 Quality control of methods

All employed methods have been tested and published by the National Center for Environment and Energy, Aarhus University (DCE). The development of methods has been done in cooperation between the Danish, German and Swedish and Finnish partners and represents state of the art in seal monitoring.

4.2 Quality control of data and reporting

Annual delivery of data and reporting of results occur to national data hosts and financing bodies. Initiatives have been taken within the HELCOM seal expert group to annually compile and secure the quality of data.

5. Contacts and references

5.1 Contact persons

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Appendix 1. Dissection protocol Reproductive health

State of decay (1-5) ☐

Number …….. **Species** …….. **Sex** ……. **Frozen** ☐ **Fresh** ☐

Body weight ……..kg **Body length** ……..cm **Anogenital distance**……..cm

Blubber chest ……..cm **Circumference** ……cm **Age** ……..

By-caught	
Found dead	
Shot	
Other	

Date of finding …… **Place of finding** ……

Finder/Correspondent ……

……

Date of necropsy …….. **Team** ……

Not whole body	

Shaded squares – prioritized samples for contaminant monitoring, *- prioritized organ weight

	Organ weight (g) (left-right)		Frozen sample "contaminant" (g)	Formaldehyde Histology	Frozen Bacteriology	Frozen Virology	Photos	Ethanol	Other
Skin									
Blubber									
Muscle									
Blood									
Serum									
Brain									
Thyroid	*	*							
Thymus									
Lung									
Heart		*							
Liver		*							
Lymph node(s)		*							
Spleen		*							
Stomach									
Small intestine									
Large intestine									
Adrenal gland	*	*							
Kidney	*	*							
Uterus									
Ovaries	*	*							
Penis									
Testicles	*	*							
Epididymis	*	*							
Prostate		*							

Number …….. Species …….. Sex ……..

Pathological findings

Blubber thickness	Chest	Stomach	Hips
Ventral (mm)			
Dorsal (mm)			

Uterus **Foetus**

Juvenile ☐ **Sex ……..**

Not pregnant ☐ **Weight ……g**

Pregnant ☐ **side …….** **Length ……cm**

Corpus albican **left ovary** ☐ **right ovary** ☐ **size ……X……mm**

Corpus luteum **left ovary** ☐ **right ovary** ☐ **size ……X……mm**

Other findings: ……

Number: The individual number given by your institution.

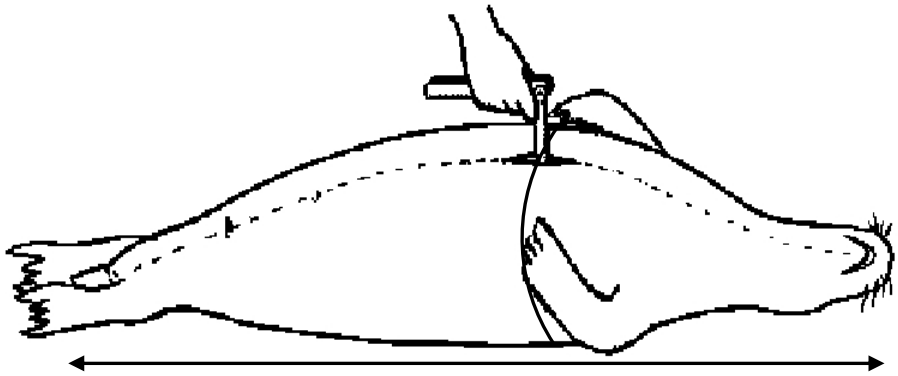
Frozen/ Fresh: Fresh means "not frozen"

State of decay:

1 fresh (only fresh, unfrozen animals)
2 beginning of decay (e.g. shot but frozen animals; frozen by-catch)
3 moderate decay (animals you could still partly sample)
4 advanced decay
5 mummified

Body weight: Measured to nearest 0.1 kg, and to nearest 0.01 in seal foetuses.

Body length: Measured from nose to tip of tail. Seal lie on back and the neck is pushed down.



Circumference: Measure the circumference just behind the hind flippers with a soft ruler or a rope that can be measured when straighten.

Anogenital distance: Measure the distance between the genital opening and anus in males.

Blubber chest: Make a cut through the skin at the central longitudinal line (crossing the circumference line) on the ventral side between the hind flippers. Measure the blubber between the skin and the underlying muscle. The skin is not included.

Organ weight

In general, clean (cut of) tissue not belonging to the organ.

Liver: Weight without the porta lymph node. Gallbladder with bile is weighted with the liver.

Heart: Make a cut through aorta and keep heart with both atriums and ventricles in one piece.

Cut through both ventricles to rinse the heart from blood. Be careful when rinsing with water and register/collect eventual parasites. Weight when rinsed.

Testicle: Testis and epididymis are weighted separately.

Ovary: Weight first, then cut and look for corpus. Corpora are measured length X breadth.

Corpus in ovary