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This revision document contains an updated version of the document 3-4 ´Guideline for monitoring reproductive status of marine mammals in the HELCOM area". All modifications are marked in tracked changes.

Background

The guidelines for monitoring reproductive status has been updated for ringed seal, harbour seal and harbour porpoise (marked changes).

Action requested

The Meeting is invited to consider/provide input for the Meeting.

HELCOM

Guideline for monitoring reproductive status of ~~seals~~ marine mammals in the HELCOM area

1. Background

The aim of this document is to define practices to monitor the indicator ''Reproductive status in marine mammals''. 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''. There are ~~three~~ four regularly occurring ~~seal~~ marine mammal 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*) and ~~.~~ harbour porpoise (*Phocoena phocoena*). In the current document, the methodology for monitoring reproductive status by necropsies ~~of seals~~ is summarized.

Female reproductive status of ~~seals~~ marine mammals is an indicator of high relevance for the management of ~~seal~~ populations and thus to the society, management and nature conservation. Changes over time in this indicator can be seen as a warning system. For instance, pathological changes of the reproduction may be caused by anthropogenic contamination of for example hormone-disrupting chemicals. Changes in reproduction success have direct influence on population growth rate and hence abundances of marine mammal populations. Thus, declines in pregnancy rates are an important part of ~~seal~~marine mammal population monitoring programs and may warrant action by the society (such as hunting regulations and further search for harmful xenobiotics/anthropogenic contaminants) to prevent negative effects and uncontrolled population declines ~~and~~ aswell as possibly adverse human health effects.

Pregnancy rate may be reduced due to the disturbance of the endocrine system that might lead to pathological changes in the uterus and the ovaries. In the Baltic seal disease complex, occlusions or stenosis of the uterine lumen was often seen in grey and ringed seals, as well as leiomyomas in the uterine wall in grey seals (Bergman and Olsson, 1985; Bergman, Olsson, 1989; Bergman et al., 1992; Bergman, 1999; Bergman et al., 2001; Bergman et al., 2003). These lesions were probably related to exposure to PCBs and frequency of these lesions in grey seals have decreased since the 1970s (Roos et al. 2012). As new contaminants are added to the already existing mixture of chemicals in the environment there is a risk of effects on the reproductive organs, but exactly how this would manifest is difficult to predict. Therefore, it is important that experienced personnel with knowledge of normal physiology and reproductive pathology perform the examinations. Pathological lesions may indicate changes in the environment, ~~or~~ emerging contaminants or diseases, or occur spontaneously at low frequency in otherwise healthy populations (for example tumors in older seals).

In the ringed seal, occlusions of the uterine horns are rare but still seen occasionally (Kauhala et al., 2018~~Reference~~). The harbor seal was not affected, but levels of PCB never reached the same high levels in the Kattegat population as in the grey and ringed seal populations in the Baltic Sea. The three species most likely have differences in metabolism and physiology that may affect the rate of metabolisation of contaminants. They have different distribution and migration patterns, different habitat use and various~~different~~ feeding habits and are consequently exposed to partly several different environmental stressors. Also, the reproductive biology differs among the marine mammal species of the HELCOM area. However, necropsies and assessment of reproductive biology parameters should be based on the same protocols and performed in exactly the same way for all ~~three~~four species. The differences in ecology and possibly physiology indicate that trends in reproductive status may have different underlying causes and could signal different stressors in the environment.

1.1 Introduction

1.1.1 Seals

- ***Pregnancy rate*** is here defined as the proportion of sexually mature females that either has a visible embryo/fetus or shows signs of a previous pregnancy. Sexually mature females can be distinguished by that they have ovulated, i.e. a *corpus luteum* (CL) has formed in the ovary. It is known that the average age at sexual maturity can change among years (Hårding and Härkönen 1995), often a result from variations in food supply. Signs of a previous pregnancy include~~s~~ a placental scar in the uterine horn and a corpus albicans (CA, the remaining structure from a degenerated CL). In the grey seal, the CA regresses during the next gestation period, and the presence of a CA in spring (before implantation period) indicates that the female was pregnant in the previous reproductive cycle or that she had an infertile oestrus cycle in the current reproductive cycle (Boyd 1982, 1984). Therefore, the presence of a placental scar should also be used for determining a pregnancy retrospectively, as the presence of a CA only may in theory overestimate the pregnancy rate.
- The ***postpartum pregnancy signs rate*** are here defined as the proportion of females with a placental scar and a CA during the period after parturition up until the expected implantation period of the next pregnancy

- The ***gestation rate*** is here defined as the proportion of sexually mature females with a macroscopically visible embryo/fetus during the period after implantation up until parturition

The postpartum pregnancy signs rate and the gestation rate are combined to calculate pregnancy rate. The postpartum pregnancy signs rate is retrospective and should be compared to the gestation period the year before (in grey seals and ringed seals). A large difference between these rates could possibly suggest an increase in fetal mortality. Neither the presence of a placental scar/CA nor presence of a fetus says anything about whether or not pregnancy was taken to term.

~~In grey seals, gestation rate is measured in females that are 6-24 years old. Postpartum pregnancy sign rate is thus calculated from females that are 7-25 years old (Kauhala et al. 2014).~~

1.1.2 Harbour porpoises

Pregnancy rate is the proportion of sexually mature females that ~~has~~ carry a visible embryo/fetus. The CA persists for a long time in harbour porpoises, making the CA only an indication of a previous pregnancy but not necessarily during the previous year. Still, presence of CL and CA are important aspects to monitor and are a part of the overall examination of the reproductive organs.

1.2 Purpose and aims

In short the monitoring of reproductive status is important to:

1. Understand population trends and abundances of Baltic ~~seals~~ marine mammals

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 blubber thickness, 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~~ information on reproduction health ~~early warning~~ of effects of new and/or old toxic substances in the environment and follow the development of known harmful substances, as well as emerging diseases or pathologies

2. Monitoring methods

2.1 Monitoring features

Pregnancy rate is measured in a~~ll three seal species~~l marine mammals in the Baltic Sea. In dead animals (hunted, by-caught or stranded), macroscopic judgement upon dissection is applied. To put the findings of the reproduction system into context a detailed pathological investigation should be conducted if possible. Tissue samples from the hunt is practical and cost-effective so it can increase the number of available samples. However, if the female is not pregnant, it may be difficult to investigate why that is, as the reason could be pathologies in tissues not provided by

the hunter. Thus, all types of sampling regimes are important to provide information to the indicator.

The age of ~~the sea~~all marine mammal species~~!~~ must be investigated. A common way to do this is to count the cementum layers of the canine tooth. In grey seals, gestation rate is measured in females that are 6-24 years old. Postpartum pregnancy sign rate is thus calculated from females that are 7-25 years old (Kauhala et al. 2014). The reason for using this age interval is that estimated age-specific birth rates increase steeply from the age of four to six (Hamill & Gosselin 1995) and decreases after the age of 24 (Bowen et al. 2006).

Ringed seals females start to ovulate around 4-5~~6~~ years of age. Postpartum pregnancy signs rate peaks at age 5–12 years and then declines, particularly after the age of 20 (Kauhala et al. 2018). Age interval for gestation rate is tentatively set as ≥6 years but no limit is set for older females.

Harbour seals ovulate for the first time when they are 3-4 years old and give birth for the first time around the age of 4-5, but after the age of 25 the fertility starts to decline (Härkönen & Heide-Jørgensen 1990). The age interval for harbour seals is set to start at 5 years old. No limit is set for older females.

Harbour porpoises reach sexual maturity between approximately 3-6 years of age (3.63 years (Lockyer and Kinze, 2003) and 4.95 (± 0.6) years (Kesselring et al. 2017). No age interval has been set for the indicator.

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. Harbour porpoises occur in the HELCOM sub basin Kattegat, Great Belt, The Sound, Kiel Bay, Bay of Mecklenburg, Arkona Basin (HELCOM 2008; assessment has not been made for the Bornholm Basin).

Grey seals give birth in late February-March. They breed during spring and after a period of delayed embryonic development, the implantation occurs during summer. A fetus is often visible from August and onward.

Ringed seals give birth in March. Similar to the grey seal, they breed ~~some time~~ after parturition and have a period of delayed implantation. Implantation occurs around mid-summer and a fetus is visible around 40 days later (Chambellant et al. 2012), in August (Swedish monitoring data).

Harbour seals give birth in June. Ovulation occurs in July (Härkönen & Heide-Jørgensen 1990) and the fetus is usually visible from October and onwards (Swedish monitoring data).

In harbour porpoises, conception takes place in August and parturition seems to peak in the summer months, particularly June (Lockyer and Kinze, 2003).

2.3 Monitoring procedure

2.3.1 Monitoring strategy

Veterinarians and specially educated biologists collect data for the indicator. The uterus is examined for a macroscopically visible fetus/embryo in seals from the post-implantation period. In seals from the preimplantation period, the uterus is examined for placental scars and the ovaries are examined for CA. In harbour porpoises, the uterus is examined for a fetus and structures in the ovaries are recorded for life history parameters. Further investigations of pathologies can be performed by established macroscopic methods within pathology as well as other commonly used techniques such as microscopy (histology-, immuno-histochemistry) and established techniques within parasitology, bacteriology and virology.

Data to be collected:

- Presence or absence of an embryo/fetus is recorded
- Presence or absence of CL is recorded, and the diameter is measured
- Presence ~~of~~ or absence of a placental scar is recorded
- Presence or absence of CA is recorded, and the diameter is measured
- Pathological changes are diagnosed (as far as possible) ~~by a pathologist based on the necropsy and histopathology.~~
- If the female is not pregnant, the suspected reason for this should be reported (if applicable)
- Age of the animal is estimated on teeth cementum analysis by counting the annual growth layers (Johnston & Watt, 1980, Lockyer et al., 2011)

Based on these variables, females can be classified as pregnant, not pregnant or unknown. In the case of declining pregnancy rates in a population, the underlying reason must be investigated. It could be related to changes in food resources, so blubber thickness is a relevant measurement to record. Further determination of ~~for example~~ e.g. infectious microorganisms or contaminants are warranted.

2.3.2 Sampling method(s) and equipment

The examination of the reproductive organs is performed according to standard necropsy procedures by veterinarians or specially educated biologists. The vagina, cervix and uterus body are cut open along the length of the organ. The uterine horns are carefully examined for occlusions/stenoses (with a probe) and carefully cut open and presence of fetal membranes, fetus, placenta/placental scars are noted. A placental scar can be seen as a dark area in the middle of the horn, which may be more or less apparent depending on the rate of healing of uterine mucosa. Each ovary is taken out of the ovarian bursa and cut into approximately 2 mm thick slices and inspected for CA and CL. All found pathologies are carefully described (and photographed) and relevant samples for further investigations are taken (e.g. samples for histology are fixed in formalin, and microbiology, toxicology and virology are frozen at -20~~8~~ or -70/80°C ~~are fixed in formalin~~). Depending on the cause of death and the time since death investigations of different detail can be performed.

Currently, a uniform necropsy protocol for seals is not used but would be sensible to establish in order to harmonize the data collection in the whole HELCOM area. For harbour porpoises, the recommendation is to use the protocol developed by ASCOBANS/ACCOBAMS (2019).

2.4 Data analysis

2.4.1 Seals

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 or calculating 3-year-moving averages is required before sample size allows statistical testing and a coarser 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 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 can be done by published standard equations, see for example Harding and Härkönen (1995).

The GES threshold for grey seals and harbour seals is set to 90% for an increasing population (Table 1). -GES in a stable and dense population has not been set. It should be emphasized that the overall trend of the indicator is important to follow.

2.4.2 Harbour porpoises

GES has not been set for harbour porpoises.

Table 1. Threshold values according to HELCOM HOD 48-2015, outcome para 3.63, Annex 4

Reproductive status of marine mammals	Species	age class [year]	pregnancy rate	Agreed on GES Agreed to publish DE: study reservation on the indicator concept and GES
	Grey seal	6	90%	
	Ringed seal (tentative)	6	90%	
	Harbour seal	5	90%	
	Harbour porpoise	?	?	

3. Data reporting and storage

Raw data and metadata should be stored in national databases and reported upon when necessary.

4. Quality control

4.1 Quality control of methods

Extensive knowledge of the anatomy, physiology and pathology of seals is essential in order to produce high-quality data. To achieve such knowledge, persons performing the examinations should be trained veterinarians or highly specialized biologists. The development of methods has been done in cooperation between the partners within HELCOM and represents state of the art in seal monitoring.

4.2 Quality control of data and reporting

As a quality control measure, each of the responsible organizations must check the gathered data for errors before reporting and data analysis.

5. Contacts and references

Responsible organizations:

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