Baltic Marine Environment Protection Commission

Working Group on the State of the Environment and Conservation

STATE & CONSERVATION 5-2016

Tallinn, Estonia, 7-11 November, 2016

Document title	Pre-core indicator 'Reproductive disorders' – proposal to shift status to core indicator
Code	4J-7
Category	DEC
Agenda Item	4J – HELCOM indicators and assessment
Submission date	14.10.2016
Submitted by	Co-Lead Country Sweden
Reference	STATE & CONSERVATION 2-2015 (paragraph 4J.9 point 22 of the outcome)

Background

STATE & CONSERVATION 2-2015 considered the indicator 'Reproductive disorders: malformed eelpout and amphipod embryos' however could not agree on the proposed GES boundary and the shift of the indicator status to core indicator at the time (paragraph 4J.9 point 22 of the outcome). It was concluded that further elaboration on how the GES boundary for amphipods other than *Monoporeia affinis* are needed, and that several countries are prepared to consider amphipod monitoring relevant for the indicator in the future.

HELCOM HOD 48-2015 agreed on a Lead Country approach for the further development of the core indicators (paragraph 3.64 of the outcome). No Lead Country has been available for the indicator, and it has been developed further by Co-Lead Countries Denmark, Sweden and Finland. Russia has also informed on expert level on available results from research projects. Estonia informed about a national project addressing malformed amphipods that could provide a relevant contribution to the indicator at HOLAS II 5-2016 (paragraph 3.6 of the outcome). Estonia informed the Lead Country Sweden that the project has focused on the species *Monoporeia affinis*.

Work to operationalize the indicator has focused on developing a balanced concept based on the available monitoring data for the relevant species. Data availability for developing an indicator evaluation for HOLAS II purposes has also been considered, and at the first meeting of the HELCOM Expert Network on hazardous substances it was concluded that it is possible for the Contracting Parties to report the needed data to COMBINE. The Co-Lead Countries have however foreseen that for HOLAS II purposes an ad hoc data call solution would be needed in order to have the indicator evaluation completed early 2017. Monitoring of hazardous substances effects on amphipods has so far most regularly been done for the key stone species *Monoporeia affinis,* which is included in the Swedish National Monitoring program since 1994. Long term field data series are lacking for other amphipod species but during the last years also other amphipod species belonging to the Gammarid group have been used for monitoring. The GES boundary proposed for these amphipod species is proposed as a Secondary GES boundary.

This document describes the GES boundary proposal endorsement for the pre-core indicator 'Reproductive disorders: malformed eelpout and amphipod embryos'.

The full indicator report is made available as background material on the State and Conservation 5-2016 meeting site.

Action requested

The Meeting is invited to:
- endorse the shift of the indicator status from pre-core to core,
- endorse the GES boundary.

Reproductive disorders: pre-core indicator GES boundary proposal

- embryo malformations of amphipods and eelpout

Good Environmental Status

The proposed GES-boundary is developed based on monitoring data, and uses the target setting approach based on percentiles of the normal distribution as the boundary. The GES-boundary is defined as a significant increase in embryo malformations compared to the background level.

The GES-boundary does not change over time; the same GES-boundary is to be applied throughout the Baltic Sea in all assessment units, as no difference in embryo malformation rate background values is detected.

GES boundary

Table 1. The GES boundary for amphipods is based on two variables, namely (1) the proportion of malformed embryos and (2) the proportion of females with more than one malformed embryo. The two variables are always to be measured simultaneously and both variables must achieve GES for an area to be considered as having reached GES. The GES boundary for the amphipod *Monoporeia affinis;*

Parameter	GES-boundary
(1) Proportion malformed and undeveloped embryos	**<5.9%**
(2) Proportion females with >1 malformed and undeveloped embryo	**<30%**

Table 2. The GES boundary for eelpout is developed based on evaluation criteria for abnormal fry development, and have been derived for evaluating occurrence of elevated levels of different types of abnormal fry development in eelpout populations.;

Parameter	GES-boundary
Malformed fry	**<2%**
Late dead fry	**<4%**
Early dead fry	**<5%**

Secondary GES boundary – Gammarids

For amphipods *Monoporeia affinis* is the most regularly used species in monitoring, and for this species the background information on the sensitivity to hazardous substances is most well developed. However *M. affinis* has very low abundance in Gulf of Finland and the Gdansk Bay. For assessment units where the species does not occur or has low abundance, a secondary GES boundary can be used if monitoring data is available for other amphipod species belonging to the gammarids.

Table 3. Secondary GES-boundary for the gammaridean amphipods *Gmelinoides fasciatus, Pontogammarus robustoides* and *Gammarus tigrinus* (based on Gulf of Finland monitoring data)

Assessment criteria	Secondary GES-boundary
(1) Proportion malformed embryos	**>5**
(2) Proportion females with >1 malformed embryo	**<20%**

Background information on the derivation of the GES boundary

Amphipods

For the amphipod *Monoporeia affinis*, data from the National Monitoring program running 1994 to 2011 were used to determine the GES-boundary for the proportion of malformed embryos (Table 4). Fourteen stations were included in the analysis. Data is based on 8,600 gravid females and 230,000 embryos (**Figure** , 2). As many samples as possible from monitoring efforts are to be included in the analysis. A higher sample size gives less variance. Since sample size varies much between years and stations, repeated hazardous sampling, i.e. Bootstrapping, was applied to control the sample size.

Evaluating if an area is in GES using amphipods is to be done by comparing detected malformations to the GES-boundary values. A sample size of 50 gravid females (about 1,500 embryos) is the recommended sample size within the Swedish National Monitoring program for evaluating the proportion of malformed embryos. The hazardous sampling of 50 females in the data set is then to be repeated 100,000 times to obtain an even spread and then the result for the location is compared to the GES-boundary values. Data from contaminated areas (outside industries) and monitoring data from reference sites is to be used to make a comparison of the proportion of females with >1 malformed embryo, to facilitate detection of statistically significant differences between pristine and contaminated areas. Hazardous repeated sampling (100 000 times) of 50 females is recommended.

Besides the malformed and membrane damaged embryos very recent results showed that also undeveloped (undifferentiated) embryos can occur due to contaminants as PAHs and zinc and this aberration is included in the analysis.

Table 4. GES-boundary for the amphipod *Monoporeia affinis,* the 90th percentile was used (Based on ICES cooperative Research Report no 315).

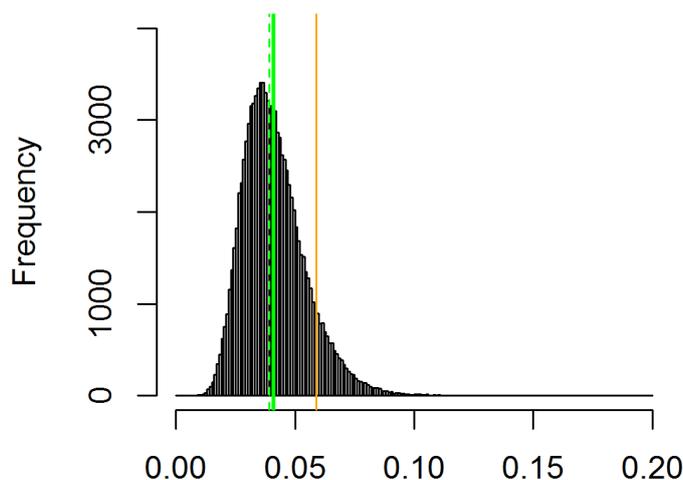| *Monoporeia affinis*	Mean	Background level (BAC)	Increased level (EAC)	**GES-boundary**
(1) Proportion malformed and undeveloped embryos	4.1 %	0-5.9 %	>5.9%	**<5.9%**
(2) Proportion females with >1 malformed and undeveloped embryo	23 %	0-30%	>30%	**<30%**

Figure 1. Proportion of malformed embryos of the amphipod Monoporeia affinis. The green line illustrates the background level, the dotted green line the median and the orange line the value that constitutes a significant increase from background, the orange line is the GES-boundary.
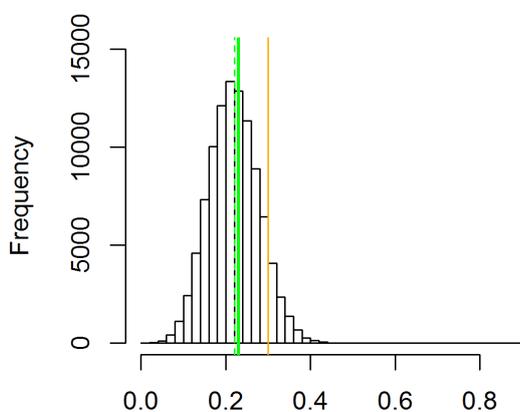

Figure 2. Proportion females with > 1 malformed embryo. Green line illustrates the mean value in background areas, the dotted line the median value and the orange line a significant deviation from background. The orange line is the GES boundary.

If the amphipod species *Monoporeia affinis* does not occur in an area, the Secondary GES boundary-Gammarids can be considered for evaluating GES (Table 5). Background assessment criteria (BAC) for other amphipods (*Gmelinoides fasciatus, Pontogammarus robustoides* and *Gammarus tigrinus*) have been derived from expert knowledge and the available mean background level of two variables, (1) the proportion of malformed embryos and (2) the proportion of females with more than one malformed embryo in the reference monitoring sites of the Gulf of Finland. EAC level was proposed if its value exceeds mean background

and significant deviation from background level (**Error! Reference source not found.**able 5). Data is based on 013-2015 monitoring at 12 sites in the Gulf of Finland.

Table 51. Secondary GES-boundary for the gammaridean amphipods *Gmelinoides fasciatus, Pontogammarus robustoides* and *Gammarus tigrinus* (based on Gulf of Finland monitoring data)

Assessment criteria	Mean	Background level (BAC)	Increased level (EAC)	GES-boundary
(1) Proportion malformed embryos	2.02	0-5	>5	**>5**
(2) Proportion females with >1 malformed embryo	14.8	0-20%	>20%	**<20%**

Eelpout

For eelpout, evaluation criteria for abnormal fry development have been derived for evaluating occurrence of elevated levels of different types of abnormal fry development in eelpout populations. The different types of abnormal fry development are early dead fry (type 0), late dead fry (type A) and malformed fry (type B-G).

Background assessment criteria (BAC) have been derived from median-values of 90th percentiles of data selected from areas regarded as less polluted reference areas in the Baltic Sea. In addition, EAC-values have been proposed as the double value of BAC, supported by a population modelling study (Bergek et al. 2012). The study shows that overall, induced malformation from environmental pollution can have a large effect on natural populations, and especially in sensitive eelpout populations with lower growth rates. Consequently, even a threshold value for EAC of 2% malformations that may seem low, could result in harmful effects at the population level (ICES WGBEC 2012).

Table 6. GES-boundary for eelpout reproductive disorders, based on mean prevalence (%) of different types of abnormal fry development (based on ICES WGBEC report 2012).

Applicable to	BAC	EAC	GES-boundary
Malformed fry	1%	2%	**<2%**
Late dead fry	2%	4%	**<4%**
Early dead fry	2.5%	5%	**<5%**

Applicability of the GES boundary

There are recognized limitations to target setting approach based on percentiles in a normal distribution. One such limitation is the assumption that the reference areas used to create the normal distribution are not affected, however for the Baltic Sea such pristine reference sites are difficult to find. Due to the lack of pristine reference sites, some uncertainty of the accuracy of the GES-boundary remains but is not deemed to be very significant due to the large amount of data available for setting the boundary. In background areas in the whole Baltic, the indicator shows similar values of embryo malformation. This means that the indicator is not sensitive to differences in salinity, depth and food availability but of course recipients with elevated concentrations of contaminants show a higher malformation rate.