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Reproductive disorders and malformed embryos of amphipods: HELCOM Supplementary indicator

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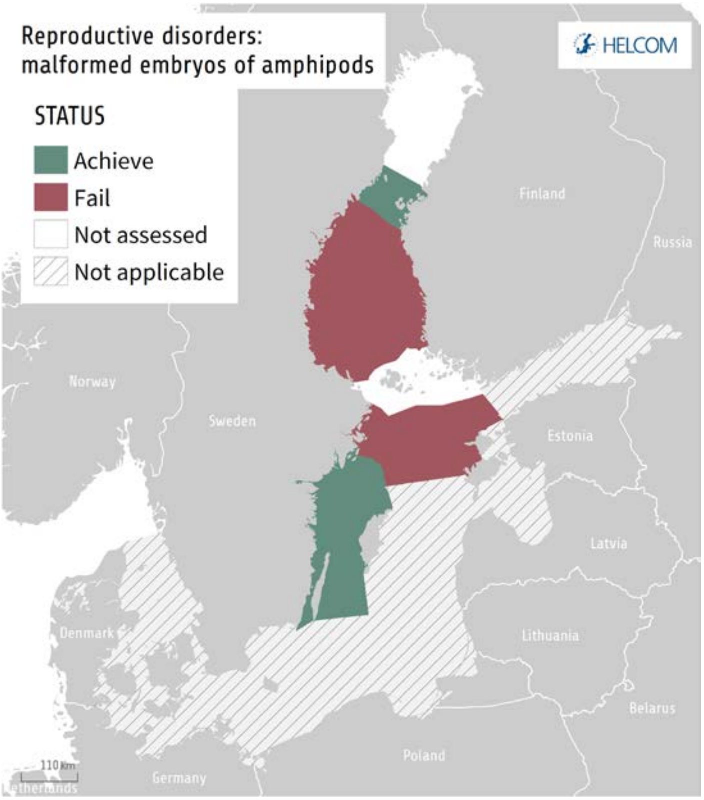
HELCOM supplementary indicator report
July 2018

Reproductive disorders: malformed embryos of amphipods

Key Message

This indicator is a HELCOM supplementary indicator and is applicable in assessment units shared by Finland and Sweden.

The rate of embryo malformations indicates reproductive toxicity due to the presence of hazardous substances in the bottom sediments. The threshold value has not been achieved at all stations within each basin, indicating that toxic effects of contaminants may be present. The variability of the malformation rate is much greater within a basin than between the Bothnian Sea and the Baltic proper.



Key message figure 1. Status assessment results are based on the evaluation of the indicator Reproductive disorders: malformed embryos of amphipods. The assessment is carried out using scale 2 HELCOM assessment units (defined in the [HELCOM Monitoring and Assessment Strategy Annex 4](#)), since no difference between the coastal and the offshore stations was found in the existing data. **Click here to access interactive maps at the HELCOM Map and Data Service: Reproductive disorders malformed embryos of amphipods**. Not applicable is used for areas in which no agreement on the application of this indicator have currently been made.

The indicator evaluation results are based on the monitoring data on malformations in *Monoporeia affinis* carried out by Sweden, with complementary data for other amphipods in the Gulf of Finland provided by Russia.

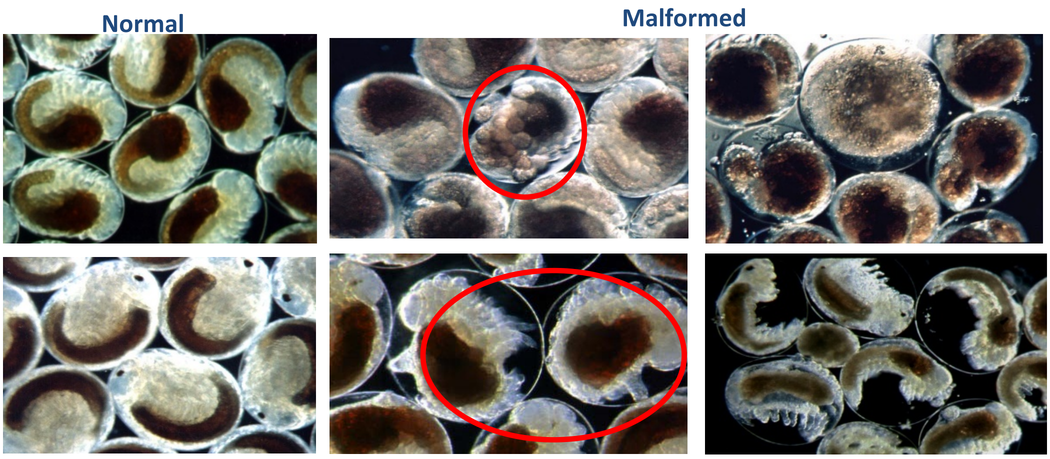
The confidence of the indicator in the assessed areas is **moderate** to **high** since more than 20 years data for setting the threshold values has been used and the assessment data are collected from multiple stations surveyed on annual basis within each assessment unit.

The indicator is applicable in the waters of Finland and Sweden.

A preliminary evaluation has been conducted in the Bornholm basin (Hano Bight) and Southern Gulf of Finland (Russia); these results however require further analysis.

Relevance of the supplementary indicator

Amphipod embryos are sensitive to sediment toxicity during the embryogenesis, and a high rate of malformations develop if the sediment where the female forages are loaded with toxic chemicals (Key message figure 2). Many chemical pollutants induce various embryo aberrations (Key message figure. 2), which makes them useful as ecologically relevant indicators for chronic biological effects of environmental contaminants. For example, in amphipods, PAHs and PCBs increase the frequency of malformed and membrane-damaged embryos (Löf et al. 2015), and the frequency of embryos with arrested development increases due to elevated concentrations of some PAHs and metals.



Key message figure 2. Normal embryos of *Monoporeia affinis* of different developmental stages (to the left) and malformed embryos (to the right).

Various amphipods and some fish species can be used for such indicator-based assessments. The amphipod *Monoporeia affinis* is a keystone species in the Baltic Sea. Its decreasing population due to impaired reproduction is a serious threat to the integrity of the ecosystem. *Monoporeia affinis* is an important food source for fish and other invertebrates; it also performs important bioturbation functions related to sediment

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oxygenation. Other amphipod species, such as *Gmelinoides fasciatus*, *Pontogammarus robustoides* and *Gammarus tigrinus*, that were used for the indicator development in the eastern Gulf of Finland are key members in the benthic communities in the coastal areas of the Baltic Sea (Gulf of Riga, Gulf of Finland, Curonian and Vistula Lagoons) and are an important prey of local fish and birds.

In the Baltic Sea, occurrence of malformed embryos in amphipods has been used as a bioindicator for reproductive toxicity caused by pollutants in benthic invertebrates for the last 20 years. The elevated frequencies of malformed embryos are regarded as a significant biological response for assessing the population-relevant effects induced by the combined exposure to the environmental contaminants in the sea sediments. The indicator provides information on the reproductive success and thereby population-persistence and stability.

Policy relevance of the supplementary indicator

	BSAP Segment and Objectives	MSFD Descriptors and Criteria
Primary link	• Healthy wildlife.	D8 Contaminants D8C2 The health of species and the composition of habitats (such as their species composition and relative abundance at locations of chronic pollution) are not adversely affected due to contaminants including cumulative and synergetic effects.
Secondary link	• Viable populations of species. • Concentrations of hazardous substances are close to natural levels.	
Other relevant legislation: WFD Biological quality (fish, benthic invertebrate, aquatic flora).		

Cite this indicator

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[Reproductive disorders malformed embryos of amphipods HELCOM supplementary indicator 2018]() **(pdf)**

Results and Confidence

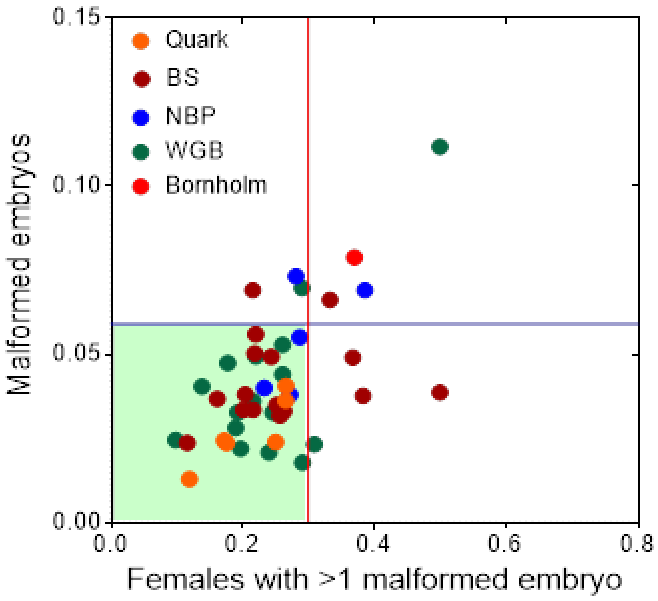
This indicator is a HELCOM supplementary indicator and is applicable in assessment units shared by Finland and Sweden.

This supplementary indicator on malformed embryos of amphipods has been applied in sub-basins shared by Finland and Sweden (the Quark and the Bothnian Sea), the Northern Baltic proper and Western Gotland Basin. A preliminary assessment was also conducted in the Swedish coastal waters of the Bornholm Basin. Although during 2011-2016 most of the observations are within the BAC values, i.e. achieve the threshold value, the deviations in both frequency of embryo malformations and frequency of females carrying malformed embryos were apparent in at least some areas of all assessment units except the Quark (Results and confidence figure 1). For comparative purposes, the indicator evaluation for Hanö Bight (Bornholm, Swedish waters; this area is known as a disturbed system, with strong indications for contaminant-driven changes) was conducted, and the results indicate high reproductive and developmental toxicity in the amphipod populations inhabiting this area (Results figure 1). For the assessment of different basins on the annual basis, a 50% rule has been applied which means that:

- each station per year was assessed individually;
- all stations grouped by regions were evaluated, and the region was considered as being in good status when >50% of the stations within the region were in good status;
- all regions within a basin were evaluated, and the basin was considered as being in good status when >50% of the regions within the basin were in good status;
- the whole six-year assessment period was considered as being in good status if >3 years were in good status.

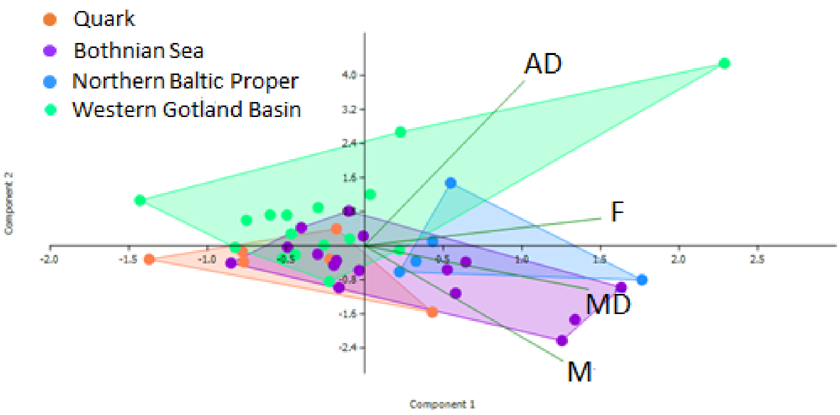
Assessment for the period 2011 to 2016 concluded that the Quark and the Western Gotland Basin have achieved good status, whereas Bothnian Sea and Northern Baltic Proper were not in good status.

The highest levels of the reproductive aberrations (frequency of females with aberrant embryos >0.4 and frequency of malformed embryos >0.06) that were observed in the evaluated units during 2011-2016 are sufficient to cause increased fluctuations in the amphipod stocks, decline in the abundance and increased probability of population decline and extinction, as suggested by population modelling of *M. affinis* (Reutgard 2015). It is unlikely that the entire Baltic population can go extinct, but decreases in local population abundance may become detrimental for the productivity of benthic communities now dominated by *M. affinis*, benthic-pelagic coupling and energy fluxes in the soft-bottom sediments.



Results figure 1. Assessment results on the performance of embryo malformations indicator), which integrates frequency of malformed embryos (Y axis) and frequency of females carrying more than one malformed embryo (X axis) in the population of *Monoporeia affinis* in 2011-2016. Blue and red lines show threshold values for the frequency of malformed embryos and frequency of females carrying more than one malformed embryo, respectively. Green shaded quartile indicates good status. Thus, observations in good status are those located in the green shaded area, and not in good status are outside of it. Each data point represents a surveyed region with several sampling stations: the Quark (1 region, 4 stations), the Bothnian Sea (BS; 4 regions, 13 stations), the Northern Baltic Proper (NBP; 1 region, 5 stations), the Western Gotland Basin (WGB; 3 regions, 13 stations), and Bornholm basin (single station in Hanö Bight); see Assessment Protocol figure 1 for sampling coverage in each assessment unit.

The relative importance of specific malformation types to the indicator-based status assessment and differences among the basins has been evaluated (Results figure 2). It was apparent that the frequencies of different aberration types vary among the basins, with arrested development being more common in the Western Gotland Basin, whereas membrane damage and complex developmental malformations of the body (limbs, eye and midgut) were more common in the Bothnian Sea and the Northern Baltic Proper. Since these malformation types were found to be specific for certain groups of contaminants (Löf et al. 2015), it would be relevant to explore these associations and integrate these data with assessment based on the contaminant analyses.



Results figure 2. Between-group Principal Component Analysis (bgPCA) evaluating relative contributions of specific malformation types (AD: arrested development, MD: membrane-damaged embryos, M: complex developmental pathologies, malformed limbs, eye and midgut) and proportion of females with more than one malformed embryo (F) to the basin-specific status of reproductive aberrations and the separation between the assessment units. Each data point is the combined value for a region, in each region, several stations were surveyed; see Assessment Protocol figure 1.

Confidence of the indicator status evaluation

The confidence of the indicator is **high** in the Bothnian Sea and Western Gotland Basin since more than 20 years of data following the same methodology for embryo analysis were used for setting the threshold values, and the 2011-2016 assessment is based on the data collected from multiple stations that were surveyed on annual basis within these assessment units. The confidence of the indicator is **moderate** in the Quark and the Northern Baltic Proper, because the spatial coverage and number of stations were somewhat lower (Results figure 1), although, considering a smaller total area of the Quark, this sampling coverage is comparable to that in the Bothnian Sea. Another area of concern with respect to the uncertainty assessment is the varying availability of the field-collected females for embryo analysis, and, thus possible inequality of the sample size between the stations/assessment units. The statistical analyses underlying indicator evaluation employ bootstrapping to generate distributions for identical sample size and derive an estimate of the confidence interval. To decrease statistical uncertainty of the analysis, several (4-8) sampling stations per assessment unit are recommended.

Future work

At present, the embryo malformation indicator has not been evaluated for all assessment units in the Baltic Sea, partly due to the lack of monitoring in these areas. The validation of the applicability of the indicator and the determination of the threshold values are needed in the Åland Sea as well as much of the eastern, south-eastern, and southern Baltic Sea before the evaluation for these areas can be conducted.

In *Monoporeia affinis* populations exposed to contaminated sediments *in situ*, frequencies of different aberration types were found to be a function of the contaminant type. In particular, occurrence of females with embryo limb malformations was strongly related to elevated concentrations of Cd and PCBs, while females with membrane-damaged embryos occurred at high PAH concentrations. Also, frequency of embryos with arrested development was positively related to levels of PAHs and metals. Thus, these reproductive aberrations can serve as contaminant-specific indicators of PCB, PAH and heavy metal exposure in biological effect monitoring. In the assessment for 2011-2016, it was found that specific aberration types contribute differently to the reproductive toxicity in the evaluated assessment units (Results figure 2).

The integration of the contaminant-specific assessment and relative frequencies for specific embryo malformations (Results figure 2) would be necessary to establish a coherent assessment for biological effects of contaminants in the Baltic Sea. Depending on the outcome of the integrated analysis of the chemical and biological data, the next step might include development of thresholds for specific malformation types. The latter would provide several additional dimensions to the multimetric embryo malformation indicator to increase its specificity toward particular contaminant types, while keeping the same concept.

Thresholds and Status evaluation

This indicator is a HELCOM supplementary indicator and is applicable in assessment units shared by Finland and Sweden.

The threshold value is established based on the monitoring data using a target setting approach with percentiles of the normal distribution as the threshold value as recommended by ICES for biological effect indicators (Davies and Vethaak 2012). The *not good status* is defined as a significant increase in embryo malformation frequency or a significant increase in frequency of females carrying more than one malformed embryo compared to the background level. The threshold values do not change over time, and the same values are proposed to be applied throughout the Baltic Sea in all assessment units because no difference in the baseline values for embryo malformation rate was detected between the basins for which the monitoring data are available.

In various Baltic Sea areas, the baseline embryo malformation levels in *Monoporeia affinis* were found to be similar to those found for the monitoring sites, implying that the indicator is not sensitive to variations in salinity, depth and food availability. At the same time, populations inhabiting areas with elevated concentrations of contaminants in the sediments or living close to known point sources showed a higher malformation rate, implying that the indicator is responsive to xenobiotics and represents a relevant measure of biological effects of environmental contaminants.

There are certain assumptions in the target setting approach when the latter is based on the percentiles of the normal distribution. One such assumption is that reference areas used to analyse statistical distribution of the malformation rate are not negatively affected and represent pristine areas. However, in the Baltic Sea such pristine areas are difficult to find. Due to the lack of unquestionably pristine reference sites, some uncertainty in the accuracy of the threshold value remains, but is not deemed to be very substantial, due to the large amount of data available for target setting and broad coverage of the area (i.e., large number of stations) within each basin.

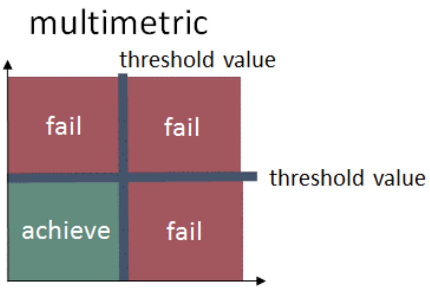
Primary threshold values

The embryo malformation indicator for amphipods is a multimetric indicator based on two variables measured in the sampled population, namely

(1) the proportion of malformed embryos and

(2) the proportion of females with more than one malformed embryo.

These two variables are measured using the same pool of field-collected gravid females; to achieve good status for an area, both variables must be below or equal their respective threshold value.

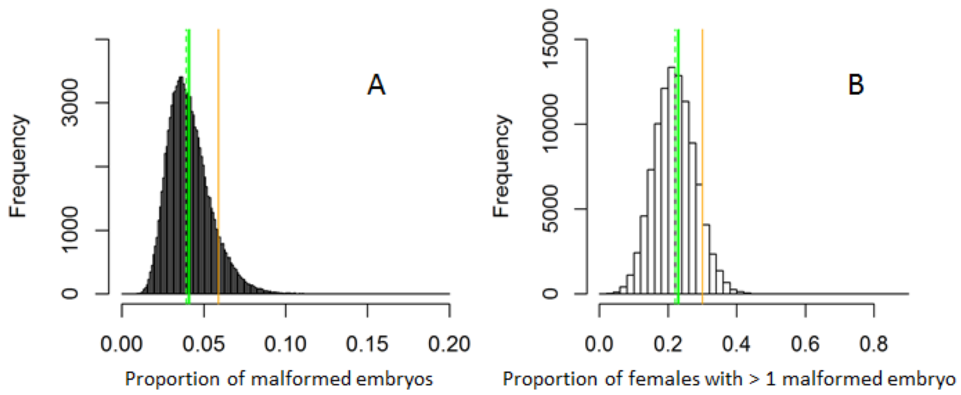


Thresholds figure 1. Schematic representation of the threshold value, which indicates good status when both the proportion of malformed embryos and the proportion of females with more than one malformed embryo are below their respective threshold values.

For the amphipod *Monoporeia affinis*, data from the Swedish National Marine Monitoring Program (SNMMP) running from 1994 to 2011 were used to determine the baselines (as mean percentage in the population) and the thresholds for the percentage of malformed embryos and the percentage of females with more than one malformed embryo (Thresholds table 1 and figure 2). Data are based on gravid females collected at fourteen stations; in total, 8,600 gravid females and 230,000 embryos were included in the analysis (Thresholds figure 2). 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, and lower bound of environmental assessment criteria (EAC) was set at the 90th percentile value for each variable (Davies and Vethaak 2012). Bootstrapping (100 000 runs) was used to derive mean, median and the 90th percentile values. All types of malformations (embryos with malformed limbs, eyes and midgut, membrane damaged embryos and embryos with arrested development) were included to the category *malformed embryos*, because all these developmental aberrations are lethal and all of them were found to be associated with various contaminants (Löf et al. 2016).

Thresholds table 1. Threshold value for the amphipod Monoporeia affinis. Background assessment criteria (BAC) and environmental assessment criteria (EAC) are adopted from Davies and Vethaak 2012.

Assessment criteria	Mean	BAC	EAC	Threshold value
Proportion of malformed embryos	0.041	<0.059	>0.059	0.059
Proportion of females with >1 malformed embryo	0.23	<0.3	>0.3	0.3



Thresholds figure 2. Defining thresholds for (A) Proportion of malformed embryos and (B) Proportion of females with > 1 malformed embryo in the population of the amphipod *Monoporeia affinis*. The solid green line indicates the background level (mean value in the population), the dotted green line denotes the median, and the orange line denotes the threshold value (90th percentile), beyond which malformation rate is significantly higher than the background levels.

Secondary threshold values - Gammarids

In areas where *M. affinis* does not occur naturally or is found sporadically and/or at low abundances, other amphipods with a similar life cycle and reproduction biology can be used to derive the embryo malformation indicator. For example, in the Gulf of Finland and the Gdansk Bay, where *M. affinis* is relatively rare, secondary thresholds were established for other amphipod species belonging to gammarids (Thresholds table 2). As with *M. affinis*, these thresholds involve two values: one for the percentage of malformed embryos and another for the percentage of females carrying more than one malformed embryo.

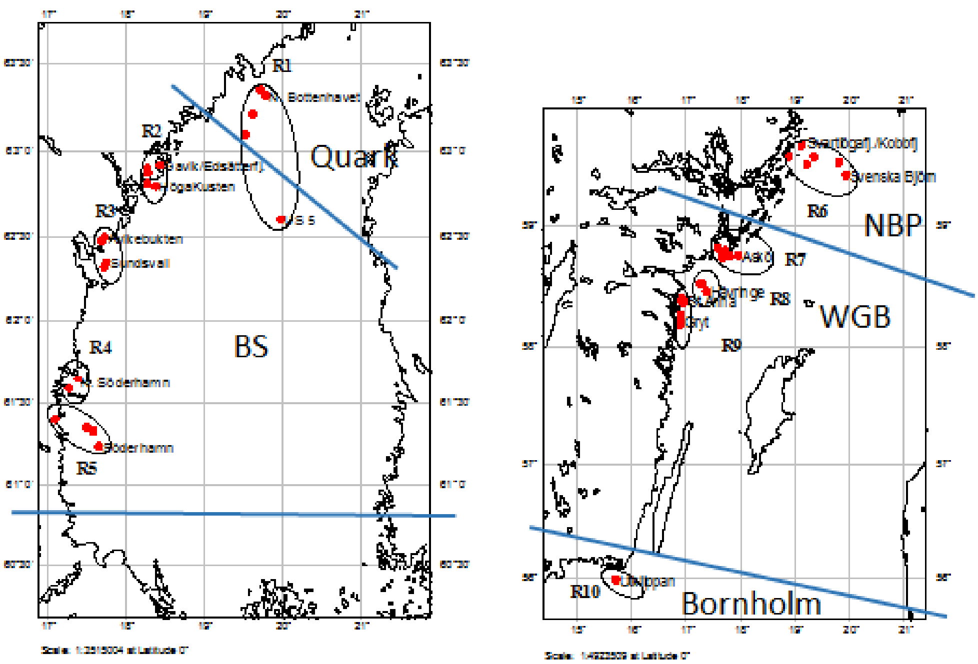
Thresholds table 2. Secondary thresholds for the gammaridean amphipods *Gmelinoides fasciatus, Pontogammarus robustoides* and *Gammarus tigrinus* (based on Gulf of Finland monitoring data, Russia).

Assessment criteria	Mean	BAC	EAC	Threshold value
Proportion of malformed embryos	0.02	<0.05	>0.05	0.05
Proportion of females with >1 malformed embryo	0.15	<0.2	>0.2	0.2

Assessment Protocol

This indicator is a HELCOM supplementary indicator and is applicable in assessment units shared by Finland and Sweden.

To decrease statistical uncertainty of the analysis, a high number of sampling stations per assessment unit is recommended. Since sample size (i.e., number of gravid females available for the analysis) may have high temporal (year-to-year) and spatial (stations within assessment unit) variability, bootstrapping can be applied to control for the sample size. For each station, a sample size of 50 gravid females and about 1,500 embryos is the recommended sample size within the Swedish National Marine Monitoring Program for evaluating the proportion of malformed embryos in the population. Depending on the area of the assessment unit, distribution of sustained populations of the species in question, and heterogeneity of hydrography, several survey regions should be included, with several stations per region (Assessment Protocol figure 1).



Assessment Protocol figure 1. Sampling design employed in SNMMP for biological effect monitoring of the amphipod *Monoporeia affinis*. Red dots denote sampling stations and ellipses are the survey regions (R1 to R10) along the Swedish coast. Basins are abbreviated as in Results figure 1. All stations are located in areas free from pollution point sources and, therefore, the indicators values are expected to be within BAC. For the assessment, a >50%-rule is applied.

Evaluating whether an area is in good status using amphipods is done by:

- Detecting the number of malformed embryos in each brood by microscopic analysis and the number of females that carry more than 1 malformed embryo; see Table 2 in Löf et al. 2015 for the description of embryo malformations and the criteria for classification of embryo pathologies;

- Calculating the percentage of malformed embryos and the number of females that carry more than one malformed embryo in the metapopulation (station); to keep the sample size (i.e. number of the females examined) constant, bootstrapping should be used;

- Combining the assessment outcome for the region if several stations per region are used; otherwise, proceed with the station-specific values;

- Comparing the detected malformation rates for the station/region to the threshold values and concluding whether both percentage of malformed embryos and the percentage of females that carry more than one malformed embryo are below their respective threshold values.

Primary threshold values for *Monoporeia affinis* has been calculated using a subset of assessment units where monitoring data for this species were available (Assessment Protocol figure 1). As no geographic difference in embryo malformation rate for the background values has been detected, the same threshold values should be applied throughout the Baltic Sea. In areas where *M. affinis* does not occur in sufficient numbers, such as the Gulf of Finland, Gdansk Basin and Arkona Basin, other amphipod species can be used, with corresponding secondary thresholds.

The indicator evaluation is done at HELCOM assessment unit Level 2, because no difference in the background values for the malformation rate between the coastal and the open sea areas was found. To evaluate the status of an assessment unit, a >50%-rule is applied, meaning that assessment unit is considered as being in good status when >50% stations with a region and >50% regions within a basin and >50% years within the assessment period are in good status. It should however be noted, that the contaminant load in sediments may vary significantly over relatively short distances and that the one-out-all-out approach may evaluate a large assessment unit as not in good status based on a station with heavily contaminated sediments. To provide a more balanced approach for such deviations and to account for a spatial variability, a region-based sampling design is recommended (Assessment Protocol figure 1); however, it is also possible that an assessment unit is represented by a single region (e.g., the Quark) sharping this case, deviations from the BAC for a single stations would be less likely to affect the assessment outcome. As some natural variation in the malformation frequencies from year to year is expected, it is recommended that the status evaluation is conducted using data for at least 3-4 years.

As pollution pressure is distinctly different in different sub-basins, evaluations made by grouping neighbouring assessment units is not deemed possible. For example, the Bothnian Bay area cannot be evaluated using monitoring data from the Bothnian Sea; hence, as no monitoring is currently carried out in the Bothnian Bay, this sub-basin cannot be evaluated.

The assessment units are defined in the HELCOM Monitoring and Assessment Strategy Annex 4.

Relevance of the Indicator

This indicator is a HELCOM supplementary indicator and is applicable in assessment units shared by Finland and Sweden.

Hazardous substances assessment

The status of hazardous substances is assessed using several core indicators. Each indicator focuses on one important aspect of the contaminant distribution, levels and effects. Currently, this biological effect indicator is not integrated to the overall assessment of contamination status; however it provides supplementary information and should be considered together with other hazardous substances core indicators in order to evaluate the overall status of hazardous substances in the Baltic Sea.

Policy relevance

The marine environment is the ultimate repository for complex mixtures of persistent chemicals. Consequently, organisms are exposed to a range of substances, many of which can cause metabolic disorders and, may affect populations through changes in growth, reproduction, and survival. The perturbations of species or communities as a consequence of exposure to hazardous substances is a prerequisite for environmental quality assessment. A reproduction indicator is important to assess integrated effect of bioavailable contaminants in the environment with strong repercussion at the population level. This goal is included in the BSAP (*Concentrations of hazardous substances are close to natural levels*) and MSFD (descriptor 8.2). Many effect variables (indicators) are not specific and respond to various environmental stressors. 'Malformed embryos' is an indicator that is comparatively specific and responds mainly to the contaminant exposure.

Role of *Monoporeia affinis and other amphipods* in the ecosystem

The amphipod *Monoporeia affinis* is a keystone species in the Baltic Sea and freshwater ecosystems below the highest coastline. It is one of the most abundant macrofauna species in soft bottoms (10 to 150 m) in the Gulf of Bothnia and the Baltic Proper, provided that oxygen conditions are sufficient (Kuparinen et al. 1996). Amphipods are very important for the oxygenation of the sediment by bioturbation (Lindström 1992), they are also food for fish, such as herring, eelpout, cod and flounder, as well as other invertebrates i.e. *Saduria entomon, Halicryptus spinulosus* and *Bylgides sarsi* (Ankar and Sigvaldsdottir 1981, Aneer 1975, Sparrevik and Leonardsson 1995). The Baltic *M. affinis* populations have decreased dramatically during the last 30 years, and currently the species is nearly absent in the Gulf of Finland and Gulf of Gdansk. The population crash in the year 2000 resulted in dramatically decreased populations in the Gulf of Bothnia (Eriksson Wiklund et al. 2008). Other amphipods used in the monitoring (*P. robustoides, G. tigrinus, G. fasciatus*) belong to so-called alien species, but they are also important components (30-40% of the total biomass) in the benthic communities in coastal areas of the Baltic Sea (Gulf of Riga, Gulf of Finland, Curonian and Vistula Lagoons) since 1990s and are the main prey for local fish and birds. These species are omnivores, with more than 50 % of detritus in their diet (Berezina 2007). These gammarids are widely used as test indicators for sediment toxicity (Berezina et al. 2017; Strode et al. 2017). All of them have a life span of 1.5 year; mating begins in April-May, embryogenesis takes 2-3 weeks, and juveniles of the 2-3 generations are released during summer (Panov and Berezina 2002; Bacela and Konopacka, 2005, Berezina et al. 2011).

Reproductive disorders in amphipods

Amphipod embryos are sensitive to sediment toxicity during the embryogenesis and various embryo aberrations can occur in response to toxic exposure. The reproductive endpoints, including embryo development are sensitive to various stress factors, including pollution. Moreover, frequency of malformed embryos is more sensitive to contaminant exposure than other reproduction variables, such as fecundity, sexual maturation and fertilization rate (Sundelin, 1983; Sundelin and Eriksson, 1998). In bioassays, exposure of *M. affinis* to metals (e.g., As, Cd, Pb) and sediments collected nearby pulp mill discharges caused higher frequencies of malformed and membrane-damaged embryos compared to reference sediments (Sundelin, 1983, 1984, 1989; Blanck et al., 1989; Wiklund et al., 2005).

All amphipod species have a similar embryo development and the method proposed here is applicable to all species. Embryo aberrations (see Table 2 in Löf et al. 2015) are classified as: (1) malformed embryo, with aberrant morphology of extremities and body symmetry (Malf), (2) embryo with damaged membrane (Membr); (3) embryo with arrested development (AD) and (4) dead or partially dead broods (DB). Categories 1 to 3 were found to be most representative of contaminant-induced developmental toxicity and these are the categories used under collective name *malformed embryos* in the indicator assessment.

Human pressures linked to the indicator

	General	MSFD Annex III, Table 2
Strong link	The most important anthropogenic threat to malformed embryos of amphipods is exposure to contaminants in the sediment.	
Weak link	Hypoxia combined to contaminants increase the incident of malformations	

The indicator is mainly sensitive to the effects of contaminants, i.e. trace metals and hydrophobic persistent organic contaminants (PHOCs). A metadata analysis for *Monoporeia affinis* at 42 sites in polluted coastal areas was used to assess the correlation between malformations and industrial waste waters. A significant relationship was found between distance to industrial outlet and malformation rate (Reutgard et al. 2014), confirming earlier studies and linking malformations to in situ exposure (Elmgren et al. 1983, Sundelin and Eriksson 1998, Sundelin et al. 2008b).

Different embryo malformation types arise in the xenobiotic-exposed females. Moreover, some types of embryo aberrations were significantly associated with specific contaminant groups in the sediment (Löf et al. 2015). In particular, occurrence of females with embryo limb malformations was strongly related to elevated concentrations of Cd and PCBs, while females with membrane-damaged embryos occurred at high

PAH concentrations. Also, frequency of embryos with arrested development was higher at elevated concentrations of PAHs and metals. Thus, these reproductive aberrations in *M. affinis* can serve as contaminant-specific indicators of PCB, PAH and heavy metal exposure in biological effect monitoring. Moreover, such aberrations as dead broods and dead eggs may indicate exposure to low oxygen concentrations during the oogenesis (Eriksson-Wiklund and Sundelin 2001, 2004). Whereas a combination of oxygen deficiency and contaminants has been observed to enhance contaminant toxicity (Gorokhova et al 2013), the oxygen deficiency as a sole factor does not give rise to malformed embryos. Food deficiency may also result in low fecundity and arrested development (Sundelin et al. 2008a), but not in other malformation types. More work is, therefore, needed to unambiguously link specific malformations to xenobiotic compounds or contaminant classes in various environmental setting.

Monitoring Requirements

This indicator is a HELCOM supplementary indicator and is applicable in assessment units shared by Finland and Sweden.

Monitoring methodology

Monitoring of embryo malformation rates in the Contracting Parties of HELCOM is described on a general level in the **HELCOM Monitoring Manual** in the monitoring topic **Biological effects of contaminants.**

However, no specific sub-programme or HELCOM monitoring guidelines have so far been developed. As the information is not yet included in the **HELCOM Monitoring Manual**, a description of the main points of the guidelines applied nationally are presented in the indicator report. Monitoring guidelines are documented in detail in 'Handboken för miljöövervakning' (in Swedish) (eng. The handbook for environmental monitoring).

The amphipod *Monoporeia affinis* and the marine amphipod *Pontoporeia femorata* are included in the national monitoring program in Sweden (SNMMP). However, *P. femorata* has very low abundance in the Bothnian Sea and the Quark. Collection of gravid females of the amphipods *Monoporeia affinis* and *Pontoporeia femorata* takes place in mid- to late January, when the embryos are in late developmental stages, which facilitates the embryo analysis. To obtain a quantitative sample, a grab sampler (e.g. Van Veen) is used to collect amphipods inhabiting sediments. When amphipods occur at low abundances, a bottom sled is used to achieve greater sampling efficiency. To collect the sediment-dwelling amphipods, 5-and 1-mm sieves could be used depending on the size of the amphipods. For species producing several broods during the reproductive period, the sampling should preferably be carried out in the early mating period when specimens in the population demonstrate a more synchronous maturation than in the later part of the reproductive period. It is also possible to collect sediment and sexually maturing females and males *in situ*, to be incubated in aquaria allowing for mating and embryogenesis. In this case, the field-collected amphipods should be transported to the laboratory in ambient water/sediment and at temperature matching their natural habitat at the time of sampling. Many Baltic glacial relicts are stenotherm cold-water species and are particularly sensitive to temperature stress during the oogenesis (Eriksson Wiklund and Sundelin 2001). During sampling of amphipods Baltic Standard is used.

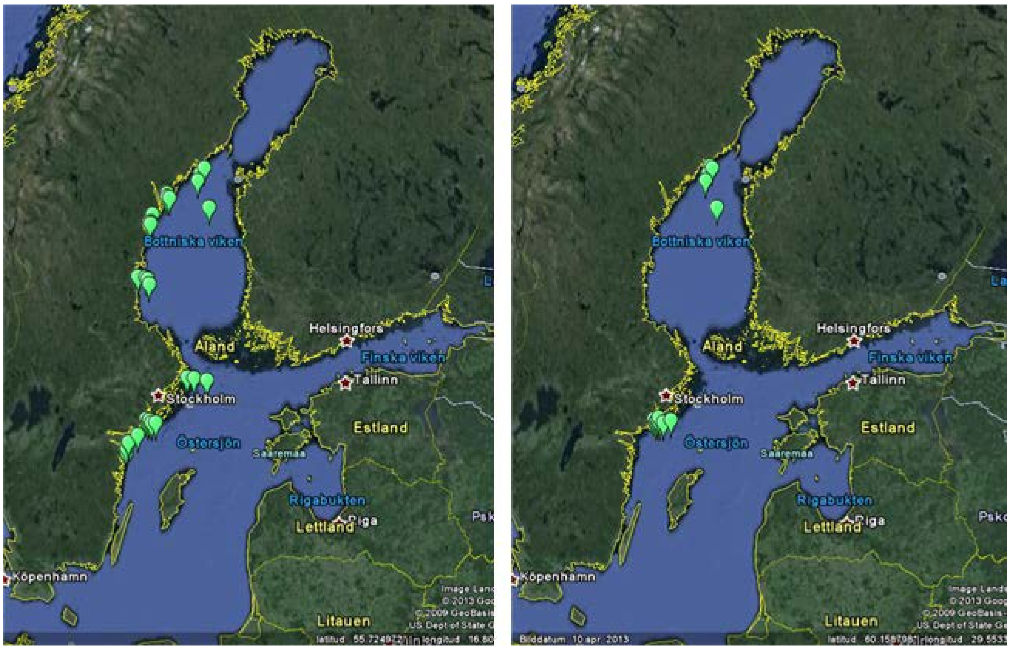
The analysis of embryos is performed on living gravid females under a stereomicroscope. The frequency of malformed embryos of *Monoporeia affinis* has a comparatively low variation in pristine areas and 50 gravid females per station give sufficient statistical power. They are analysed for fecundity in terms of number of eggs per female, number of malformed, membrane damaged embryos, dead embryos and females with dead broods. For details see Sundelin et al. 2008 (Times no 41, http://ices.dk/publications/our-publications/Pages/-ICES-Techniques-in-Marine-Environmental-Sciences-.aspx). When conducting embryo analyses, several persons analyse the same brood to assess the accuracy of determination. Normally there is 95-98 % agreement between the experts.

The amphipods used for the secondary threshold are *G. tigrinus*, *G. fasciatus* and *P. robustoides.* These species were used as bioindicators in survival test within Russian Research Monitoring Programs by different institutions from 1990s, and monitoring of amphipod reproduction started in 2009. This method is developed mainly by the Zoological Institute of the Russian Academy of Sciences. Preliminary assessment of environmental health in the eastern Gulf of Finland was conducted at 12 coastal sites and is planned to continue. Collection of these amphipods is conducted from end of May to September by a grab or scuba diving. The field-collected living amphipods are transported carefully to the laboratory and analysed under stereomicroscope. The amphipods are separated into four categories: juveniles, males, females I (without eggs in marsupium) and females II (gravid females, with eggs/embryos in marsupium). The determination of the reproductive status of the females is based on the presence and structure of brood plates (oostegites). The immature females have oostegites lacking setae. The gravid females are size-sorted in a 0.5 mm step and placed individually into Petri dishes; then the eggs are carefully teased out of the marsupium with pins or forceps. Only females carrying a closed marsupium are included in the analysis. The seven developmental stages of eggs (embryos) may be distinguished according to Weygoldt (1924) and Scadsheim (1982), cited in Pöckl (1993).

Current monitoring

The indicator on malformations in amphipod embryos has been monitored in the SNMMP since 1994 at 14 stations in Bothnian Sea and northern Baltic proper, in 2012 the program design was changed to include more stations to give a more comprehensive covering of the Baltic (Monitoring requirements figure 1).

Monitoring of amphipod reproduction in the Gulf of Finland started in 2009 and is conducted by the Zoological Institute of the Russian Academy of Sciences.



Monitoring requirements figure 1. Sampling stations used for amphipod collection in 1994-2011 (left) and from 2012 onwards (right) within the Swedish National Marine Monitoring Programme (SNMMP).

Description of optimal monitoring

Monitoring of embryo malformation rates should be carried out in all relevant HELCOM assessment units. Extending the spatial scope of the monitoring effort would increase the possibility to accurately evaluate the pressure from environmental contaminants on benthic animals in the Baltic Sea. To obtain a more comprehensive picture of the health status of the amphipods, it would be optimal to include also stations in the Bothnian Bay, southern part of the Baltic, including Bornholm. Particularly alarming situation in the Hanö Bight has been found by the surveys conducted during the assessment period. Recently Estonian scientists have started to monitor *Monoporeia affinis* in coastal areas. Also, in the Gulf of Riga, scientists started to work with embryo malformations of amphipods including *M. affinis.*

Data and updating

This indicator is a HELCOM supplementary indicator and is applicable in assessment units shared by Finland and Sweden.

Access and use

The data and resulting data products (tables, figures and maps) available on the indicator web page can be used freely given that the source is cited. The indicator should be cited as following:

HELCOM (2018) Reproductive disorders: malformed embryos of amphipods. HELCOM supplementary indicator report. Online. [Date Viewed], [Web link].

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Metadata

Result: Reproductive disorders: malformed embryos of amphipods
Data: Reproductive disorders: malformed embryos of amphipods

Data host for Swedish amphipod data is IVL but from 2018 SGU (Geological Survey of Sweden) is data host. Project data from BONUS project BEAST are stored in the BONUS haz database hosted by Aarhus University in Denmark. Data are under development to be delivered to ICES. Project data from BONUS project BEAST is also stored in the BONUShaz database hosted by Aarhus University in Denmark.

Contributors and references

This indicator is a HELCOM supplementary indicator and is applicable in assessment units shared by Finland and Sweden.

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Archive

This version of the HELCOM supplementary indicator report was published in July 2018:

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Earlier versions of this indicator are available at:

HOLAS II component - Core indicator report – web-based version July 2017 (pdf)

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