Integrated Workflow for Assessing Biological Effects of contaminants in the Baltic Sea

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Overview of iBEC

iBEC (integrated Biological Effects of Contaminants) is a systematic and reproducible workflow designed for analyzing biological effects to assess environmental stress, particularly from contaminants. It covers both suborganismal (e.g., biochemical and cellular) and organismal (e.g., reproduction and growth) responses, aiming to offer a comprehensive biological effect assessment for Descriptor 8.2 (D8.2) under the EU Marine Strategy Framework Directive (MSFD). The applicability of the workflow is demonstrated for the Bothnian Sea and Gulf of Riga using the data compilation provided by the BEACON and H-BEC projects. By streamlining the data management process, iBEC facilitates the efficient and accurate assessment of environmental stressors on marine life, supporting both regulatory compliance and scientific research.

Key Points of iBEC:

1. **Scope and purpose**: iBEC is designed to collate and analyze biological effect measurements for assessing contaminant exposure in marine environments. Specifically developed for D8.2 implementation in the Baltic Sea, iBEC employs local species suitable for evaluation of environmental stress from pollutants in this ecosystem with high environmental variability and species-poor communities.
2. **Biological effects are measured** at different levels of biological organisation. Suborganismal responses include biochemical, cellular, and molecular markers that indicate stress at a microscopic level. Organismal responses encompass metrics like reproduction and growth, providing a broader picture of organism health. iBEC includes biomarker responses and physiological health parameters that have been tested in the Baltic biota and found informative.
3. **Applications**: (1) **Environmental contaminant assessment** ato support D8 evaluation by specifically targeting biological responses to pollutants (D8.2); (2) **Ecotoxicological surveys** in the field and laboratory settings to understand and predict pollution impacts.
4. **Data organization**: Information is systematically organized in a series of spreadsheets, which helps manage and analyze available datasets. The spreadsheets are complemented by a separate *Standardized and automated spreadsheet* provided by Ifremer (France) for calculating the data distribution for biological effect parameters in relation to the background variability at each station and visualization of the assessment outcome. Other

additional files inlude data used for case studies providing the example assessments.

5. **Data requirements for D8.2 assessment**: To ensure consistency and reliability, criteria for data collection and principles of data aggregation across stations and years to represent subbasin status over defined time periods (e.g., 6 years for HOLAS) are defined.

Background

Assessment needs

The traditional approach of assessing chemical pollution through single-chemical concentrations is increasingly questioned. Measuring pollutant concentrations in seawater has its advantages, such as the ease of conducting targeted analyses and the ability to directly link results with contamination sources. However, there are also drawbacks, including the challenge of detecting low concentrations, the influence of random spatial and temporal variations, and the potential oversight of factors like bioavailability, mixture effects, and varying environmental conditions. The Descriptor 8.2 allows for the inclusion of biological effects in assessments of contaminant impacts to determine if harm is occurring. This requires biological effects data and an integration system o evaluate the environmental status of contaminants (ICES, 2021). OSPAR Convention (Convention for the Protection of the Marine Environment of the North-East Atlantic) and JAMP (Joint Assessment and Monitoring Programme) have recommended a set of biological effect techniques and assessment criteria (JAMP, 2012) as well as the integrated approach developed over decades (Vethaak et al., 2017). Several national and international projects have concurrently assessed the potential and benefits of biological effect monitoring and assessment in the Baltic Sea, offering robust recommendations to complement current contaminant effect assessments led by HELCOM. These project outcomes have been instrumental in developing the iBEC for enhanced environmental monitoring and D8 assessment.

JAMP/OSPAR approach

The iBEC concept is based on the existing multistep data aggregation approach to assess contaminant and biological effects data together; the approach is advocated by the OSPAR and JAMP. Within this framework, Background Assessment Concentrations (BAC) and Environmental Assessment Criteria (EAC) are key tools used to evaluate the levels of contaminants and their potential impacts on the environment and organisms:

- **Background Assessment Concentration:** BAC values represent the baseline condition of a contaminant or biological marker in the environment to provide a benchmark against which observed data from potentially contaminated sites can be compared. They are usually determined through the analysis of long-term environmental monitoring data to understand the natural variability of contaminant levels and biomarkers. A statistical approach, using either the lower or the upper (depending on the effect direction) bound of the 90% confidence interval, is employed to establish the BAC value, ensuring that it reflects the upper/lower limit of the natural variation.
- **Environmental Assessment Criteria:** EAC values are thresholds used to assess the potential risk or harm that contaminants may pose to biota. They represent biomarker levels above which there may be adverse organismal effects. EACs are typically derived from ecotoxicological studies, including laboratory toxicity tests and field observations, as well as species sensitivity distribution (SSD) models, and are used to guide regulatory

actions. To account for uncertainties and variability, safety factors are applied to toxicological thresholds such as NOEC (No Observed Effect Concentration), LOEC (Lowest Observed Effect Concentration), LC50 (Lethal Concentration for 50% of the population), and EC50 (Effective Concentration for 50% of the population).
- **Risk identification:** If observed levels exceed BAC but are below EAC, further investigation may be warranted. When observed levels exceed EAC values, there is a clear indication of potential adverse effects, prompting risk management actions.

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Baltic biota: selecting relevant target species and markers

Biological responses to toxic substances vary across different levels of organization, from molecular to ecosystem scales. Molecular and cellular responses are highly sensitive and specific to particular toxins, but their ecological significance remains unclear. Responses at higher levels, such as changes in population abundance or biodiversity, are ecologically relevant but cannot always attribute differences solely to pollutants. Balancing these factors is crucial for effective environmental monitoring, aiming for sensitivity as an early-warning signal, ecological relevance in terms of population fitness, and practicality in terms of standardization and cost-effectiveness.

The Baltic Sea presents a unique and challenging environment for biological assessments due to its variable hydrographic conditions and strong environmental gradients. Selecting relevant target species and biomarkers for monitoring contaminant impacts requires careful consideration of these factors (Table 1). To ensure comprehensive and reliable assessments, target species and biomarkers in the Baltic Sea must account for strong environmental gradients, benthic-pelagic coupling, physiological sensitivity to stressors, and representative species distribution.

Also, the existing monitoring of the Baltic environment guided by HELCOM is crucial because it ensures consistent data collection and comparability across different regions. This monitoring provides comprehensive datasets that reflect the overall status of the Baltic Sea ecosystem, aiding in the identification of trends and the assessment of long-term impacts from contaminants and other stressors. Therefore, selecting target species for biological effect assessment should align with existing monitoring efforts to enhance data collection and optimize the use of results.

As indicated by our inventories, pertinent target species, such as fish (perch, herring, eelpout) and invertebrates (amphipods, blue mussel, clam), have been validated for monitoring contaminant impacts in the Baltic Sea. Several biomarkers have been established and tested across these species in various research projects, demonstrating their applicability for long-term monitoring. Integrating sensitive subcellular responses with organismal indicators, such as changes in reproductive success, growth, and physiological indices, ensures early detection of environmental threats and identification of the severity of adverse effects.

iBEC description

Overall structure

iBEC is a workflow developed by the BEACON (Interreg project; 2023-2024) to organize, collate and analyze biological effect measurements for assessing contaminant exposure in the context of D8.2. The workflow is organized in *iBEC tool.xlsx* file comprised a set of spreadsheets and an additional *Standardized and Automated Spreadsheet* provided by Ifremer. **The primary output** is an evaluation of the environmental status that integrates multiple lines of evidence for the biological effects of contaminants reflecting *in-situ* exposure to the ambient contaminant mixtures and under existing environmental conditions.

Information in the spreadsheets

Each spreadsheet within the *iBEC tool.xlsx* is dedicated to specific details concerning sampling sites, species, markers, and the required BAC/EAC values as well as the assessment output. All fields in the spreadsheets are color-coded to indicate their usage guidelines. Grey fields are designated for manual data entry only, while all other fields should either be populated by copying data from other spreadsheets (green fields) or calculated automatically (blue fields). Yellow and purple fields contain information that should not be changed for each assessment but, if needed, can be revised (e.g., when the approach is developed further). In addition to the spreadsheets needed for the analysis, a set of examples for the primary data structure is provided; these example are located as the last spreadsheets in the *iBEC tool.xlsx*.

The following spreadsheets are included:

- **Assessment Units** {fixed data for all assessments}: HELCOM Assessment Units, Scale 2.
- **Stations** {input data}: List of stations with their geographical coordinates for each sub-basin included in the assessment. Add stations as needed but check first for the existing station coding and spelling in national register and/or other databases (e.g., ICES DOME).
- **Species** {input data}: List of species used in the assessment. The list of species can be extensive, and not every species is required for each assessment. However, at least two species from each basin are necessary to ensure a valid assessment.
- **Sex** {input data}: Sex of the test animals (*male/female*) must be specified as many biomarkers have sex-specific BAC values. For juveniles in many species, however, the sex is often not feasible to determine (*unknown*). If individuals of different sex are mixed in a composite sample (*mixed*), the variability for a response variable should be calculated using relative proportion of males and females in the sample.
- **Tissues** {input data}: List of tissues used in the analysis.
- **BE Rationale** {modify if necessary}: List of Biological Effect (BE) parameters included in the assessment with corresponding units and rationale for their interpretation. This list can be extensive, and not every biomarker/physiological variable is required for each assessment. However, at least two exposure and two effect markers should be included to ensure a valid assessment.
- **TV** {input data on Target Values}: Exposure and effect biomarkers used for each species and their target values estimated using the BAC/EAC approach (i.e., 90%-confidence interval with non-parametric bootstrapping for 1000 observations based on measurements from reference sites). At least five observations for each BE parameter are required for a

valid assessment.

- *Standardized Automated Spreadsheet* {a separate file} for evaluating data distributions. Currently, identical to the Ifremer template. For each BE parameter/species/station, primary data are used to calculate whether the observed mean value is deviating significantly from the respective BAC/EAC value.
- **Data Summary** {input data}: Summary of the data for the BE parameters and target species used for the assessment. Data are entered as mean values for each BE parameter/species/station. For MSFD assessment (a 6-year cycle), at least three years of observations are required.
- **BAC Exceedance** {input data using the output generated by Standardized Automated Spreadsheet}: Comparing observed vs target values for each BE parameter and scaling to 0/1 [0: exceeding BAC/EAC (=sub-GES), 1: not exceeding BAC/EAC (=in-GES)].
- **Assessment** {output data}: Assessment summary for each station and the entire basin based on the percentage of BE parameters exceeding their respective BAC/EAC values.
- **Visualization** {output data}: A diagram summary for each station and the entire basin based on the assessment outcome (under construction).

Data processing: step-by-step guide

Step 1: For each subbasin, provide list of stations with geographic coordinates and auxilliary information (->Stations).

Step 2: Provide list of species used for BE analysis with auxilliary information (->Species).

Step 3: Examine the list of BE parameters to confirm that it contains all variables used in the analysis; add new variable only if necessary (->BE Rationale).

Step 4: For each subbasin, select species, tissue and, if needed, sex for each BE parameter analyzed (->TV).

Step 5: For each subbasin, insert species-, tissue- and, if needed, sex-specific BAC/EAC values as TV (target values) for each BE parameter (->TV). See section X for the guidelines on setting BAC/EAC values.

Step 6: Prepare your primary data for BE parameters in a separate file (see Example_data); check for outliers and ensure that the measurement units are correct (i.e., they are identical to those in BE Rationale). Calculate average values for each BE parameter/station and transfer these values to Data Summary.

Step 7: Open *Standardized Automated Spreadsheet* and insert the BAC/EAC values for each BE parameter/station as well as raw data to compare whether the observed data differ from the corresponding BAC/EAC values. This assessment is station-based; therefore, if several stations are used to assess a subbasin, each should be evaluated separately.

Step 8: Transfer the outcome of the station-based assessment (->BAC exceedance), using 0/1 coding to provide an overview of the BE parameters exceeding and not exceeding thier BAC/EAC values.

Step 9: Complete the integration of the station-based assessments and aggregation at the subbasin level (->Assessment).

Step 10: Visualize the assessment outcome and provide a short interpretation (->Visualization).

Examples of biological effect assessment in different subbasins

Bothnian Sea

Data source: text

Target species and BE parameters:

Primary data file: filename

BAC/EAC setting: text, filename

Assessment outcome: text

Gulf of Riga

Data source: text

Target species and BE parameters:

Primary data file: filename

BAC/EAC setting: text, filename

Assessment outcome: text

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