Status for biological effect indicators monitored in Danish marine ecosystems

Scientific briefing from DCE – Danish Centre for Environment and Energy Date: 17 November 2021 | **59**

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Data sheet

	Scientific briefing from DCE – Danish Centre for Environment and Energy
Category:	Scientific briefing
Title:	Status for biological effect indicators monitored in Danish marine ecosystems
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Referee: Quality assurance, DCE: Linguistic QA:	Martin Mørk Larsen Susanne Boutrup Charlotte Kler
External comment:	The Danish Environmental Protection Agency. The comments can be found here: http://dce2.au.dk/pub/komm/N2021_59_komm.pdf
Claimant:	Ministry of Environment
Please cite as:	Tairova Z., Strand J. 2021. Status for biological effect indicators monitored in Danish marine ecosystems. Aarhus University, DCE - Danish Centre for Environment and Energy, 34 s. – Scientific briefing no. 2021|59 https://dce.au.dk/fileadmin/dce.au.dk/Udgivelser/Notater_2021/N2021_59.pdf
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Front page photo:	Gammarus spp. (photo Z.Tairova)
Number of pages:	34

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Preface

This scientific briefing has been prepared based on an order from the Ministry of Environment with the assignment of assessing pending issues for biological tests, up to the point when Denmark will have fully functional biological effect indicators for use in monitoring the marine environment.

This has been done by preparing a status assessment of relevant criteria/elements that are recommended for internationally established monitoring indicators for the marine environment.

Introduction

Since 1998, different types of biological effect indicators for pollution effects related to contaminants have been included in the marine part of the National Monitoring Programme for the Aquatic and Terrestrial Environment (NOVANA) in Denmark (Miljøstyrelsen et al., 2017).

Some indicators are also included into the Danish Marine Strategy (Miljøstyrelsen, 2020), as part of Descriptor 8 – Contaminants (D8C1, D8C2). Decriptor 8 is defined: "Concentrations of contaminants are at levels not giving rise to pollution effects" (EC JRC, 2015).

In the current document, the employed indicators are assessed using the list of coordination tools, which, according to international monitoring programmes, should preferably be in place in order to be applicable as functional indicators in environmental assessment. These coordination tools include: monitoring guidelines/monitoring methods; quality assurance procedures; quality control; assessment criteria; database; temporal and spatial scope.

Monitoring data for biological effect indicators can be used for both state and impact assessments and they can also be evaluated using environmentally relevant assessment criteria (ACs) developed within international scientific fora working with monitoring and assessment. Several of these ACs have also been adopted for indicator assessments by The Baltic Marine Environment Protection Commission – also known as the Helsinki Commission (HELCOM), The Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) and reported by the International Council for the Exploration of the Sea (ICES).

For a range of biological effect indicators, background levels have been set for the biological responses, which correspond to the upper limit for the variation in a background assessment criteria (BAC) and, for some indicators, the environmental assessment criteria (EAC) have been established as well. BAC is analogous to background assessment concentrations, or a natural response level, and EAC represents levels of response below which unacceptable responses at higher, e.g. organism or population, levels would not be expected (Davies & Vethaak, 2012; OSPAR, 2013).

These ACs have also been developed for the indicator organisms and the respective biological effect measurements deployed within the NOVANA-programme, i.e. covering fish, mussels, marine gastropods and amphipods (Table 1).

Table 1. Biological effect measurements covered in the NOVANA-programme.

Indicator species	Type of indicators	Indicator name
Fish (eelpout)	PAH-specific effects	CYP1A (EROD) activity and PAH-metabolites (FAC)
	General pollution effects	Reproductive success
Mussels (blue mussel)	General pollution effects	Lysosomal membrane stability
Marine gastropods	TBT-specific effects	Imposex and intersex
Amphipods	General pollution effects	Reproductive success

This overview report focuses on indicators that are employed in NOVANA, but also ACs for other indicator species and biological effect measurements are included in supplementary tables. These alternative ACs are taken from the Report of the Working Group on the Biological Effects of Contaminants (WGBEC) and those that are currently included in OSPAR's Coordinated Environmental Monitoring Programme (CEMP) (ICES WGBEC, 2013; OSPAR CEMP, 2021).

CEMPs' objective is to deliver comparable data from across the OSPAR Maritime Area that can be used in assessments to address the specific questions raised in OSPAR's Joint Assessment and Monitoring Programme, (JAMP). Certain indicators, where monitoring guidelines, quality assurance tools and/or assessment tools are currently lacking, have been categorised as 'Pre-CEMP'. Monitoring of these components is voluntary on a temporary basis, pending the development of those requirements (OSPAR CEMP, 2021).

The ACs for polycyclic aromatic hydrocarbons (PAHs), metal-specific and general biological effects are currently included in the pre-CEMP category (*Supplementary tables, S5, S6*).

The following ACs reported in this overview are the current criteria applied within the NOVANA-programme and are based on outcomes from WGBEC, HELCOM Indicator factsheets and OSPAR CEMP.

Fish, eelpout (*Zoarces viviparus*)

In NOVANA's fish component, the following effect indicators are currently monitored:

"CYP1A enzymatic activity (EROD)": OSPAR JAMP technical annex 2 on PAH-specific biological effects monitoring, although also currently under (OSPAR pre-CEMP: "*general biological effect*"). This indicator was also part of the HELCOM candidate indicator list, but it was not anticipated to be ready for use in HOLAS II (HELCOM HOD, 2016).

The activity of the cytochrome P450 1A (CYP1A) family of enzymes that is responsible for primary metabolism of PAHs and polychlorinated biphenyls (PCBs) is measured using artificial substrate 7-ethoxyresorufin (EROD). PAHs, PCBs and dioxin-like compounds induce synthesis of CYP1A by binding to aryl hydrocarbon receptor/ARNT complex and, thus, the measurement of the EROD activity is used as a tool to measure the induction of this enzymatic system caused by this type of pollution (Davies & Vethaak, 2012). The BAC-value for eelpout in Table 2 is reported in ICES WGBEC report (2013) and OSPAR (2013).

In addition, the WGBEC and OSPAR pre-CEMP categories include ACs for several other OSPAR relevant fish species (Tables S1 and S2, Supplementary Tables).

Table 2. Assessment criteria for biological effect indicator, " CYP1A enzymatic activity (EROD)" in eelpout, used in the Danish monitoring programme NOVANA (ICES WGBEC, 2013; OSPAR, 2013).

Effect indicator	BAC	EAC
Eelpout		
CYP1A-enzymatic activity (pmol min^{-1} mg $protein^{-1}$)*	*10*	-

Notes:

* measured in S9-fraction of liver tissue extract

In the NOVANA programme, the following components, necessary for monitoring application, are developed or used:

- monitoring guidelines / monitoring methods are in place (Strand, 2013a based on Stagg & Mcintosh, 1998, and revised in Ron Stagg et al., 2016);
- procedure for quality assurance of the method: participation in international laboratory intercalibration exercises and workshops (Förlin, 2012; BEQUALM);
- procedure for quality control of monitoring data by the Marine Topic Center, Aarhus University (Høgslund et al., 2019);
- assessment criteria – Table 2;
- monitoring databases – national database MFS-base/HSD database (data from MFS-base will be transferred to a new monitoring database at the Danish EPA); international database – ICES DOME;
- temporal and spatial scope – included in the NOVANA programme since 2004: within the period of 2017-2021, 2 stations are monitored every year (Miljøstyrelsen et al., 2017).

"PAH-metabolites in bile" (OSPAR JAMP and pre-CEMP: "*PAH specific biological effect*"; HELCOM Core indicator: "*Polyaromatic hydrocarbons (PAHs) and their metabolites*").

The metabolites of PAHs are present in bile at the final stage of the biotransformation process through which lipophilic compounds are changed into more water-soluble forms and then excreted from the organism. Therefore, measurement of these compounds in fish bile, using fluorescence analytical techniques, is used as a tool to assess the exposure of fish to PAHs (Davies & Vethaak, 2012). The ACs for eelpout reported in ICES WGBEC report (2013) and OSPAR (2013) are in Table 3.

In addition, the WGBEC and OSPAR pre-CEMP categories include ACs for several other OSPAR relevant fish species (Tables S1 and S3, Supplementary Tables). Additionally, the EAC for Cod (483 ng g^{-1}) has a potential to be used in the Danish monitoring programme as AC for eelpout, since this value is regarded as the most relevant for fish species for Danish waters.

Table 3. Assessment criteria for biological effect indicator "PAH-metabolites in bile" in eelpout used in the Danish monitoring programme NOVANA (ICES WGBEC, 2013;OSPAR, 2013).

Effect indicator	BAC	EAC
Eelpout		
PAH metabolites in bile (ng ml^{-1})	*92*	*(483)**

Notes:
*Adopting the Cod EAC value (ng g^{-1}), GC/MS data directly compared to HPLC/F data (ng ml^{-1}).

In the NOVANA programme, the following components, necessary for monitoring application, are developed or used:

- monitoring guidelines / monitoring methods are in place (Strand, 2013a; Ariese et al., 2005, based on OSPAR Commission, 2008, Annex 2);
- procedure for quality assurance of the method: participation in international laboratory intercalibration exercises and workshops (Förlin, 2012; Kammann et al., 2013; BEQUALM);
- procedure for quality control of monitoring data by the Marine Topic Center, Aarhus University (Høgslund et al., 2019);
- assessment criteria – Table 3;
- monitoring databases – national database MFS-base/HSD database (data from MFS-base will be transferred to a new monitoring database at the Danish EPA); international database – ICES DOME;
- temporal and spatial scope – included in the NOVANA programme since 2004: within the period of 2017-2021, 2 stations are monitored every year (Miljøstyrelsen et al., 2017).

"Reproductive success in eelpout" (*General biological effect*, most recent ACs in WGBEC 2012, OSPAR 2013). This indicator was proposed as a pre-core indicator in the HELCOM indicator list, but it was not anticipated to be ready for use in HOLAS II (HELCOM HOD, 2016). This indicator was included in JAMP Guidelines for General Biological Effects Monitoring (OSPAR, 2007).

Reproductive success is measured as proportion of malformed fry (types B-G), proportion of late dead fry (type A), proportion of early dead fry (type 0)

and proportion of total abnormal fry (all types). The eelpout (*Zoarces viviparus*), also called viviparous blenny, can be used as a bioindicator of the impact of hazardous substances on reproductive success of viviparous fish in the marine environment. The hazardous substances that can affect embryo and larval development in fish include organochlorines, pesticides, polycyclic aromatic hydrocarbons (PAHs), heavy metals and organometals (Davies & Vethaak, 2012). The ACs for eelpout reported in ICES WGBEC report (2013) and OSPAR (2013) are listed in Table 4.

Table 4. Assessment criteria for biological effect indicators in eelpout for "Reproductive success in eelpout" used in the Danish monitoring programme NOVANA (ICES WGBEC, 2013; OSPAR, 2013).

Effect indicator	BAC	EAC
Eelpout		
Mean prevalence of malformed fry (type B-G)	1%	2%
Mean prevalence of late dead fry (type A)	2%	4%
Mean prevalence of early dead fry (type 0)	2.5%	5%
Mean prevalence of total abnormal fry (all types)	5%	10%

In the NOVANA programme, the following components, necessary for monitoring application, are developed or used:

- monitoring guidelines / monitoring methods are in place (Strand, 2013a, based on OSPAR, 2007, Annex 10 and Strand et al., 2004);
- quality assurance procedures: participation in international workshops with practical exercises (Förlin, 2012);
- quality control on monitoring data is in place under the Marine Topic Center, Aarhus University (Høgslund et al., 2019);
- assessment criteria – Table 4;
- monitoring databases – national database MFS-base/HSD database; international database – ICES DOME;
- temporal and spatial scope – included in the NOVANA programme since 2004: within the period of 2017-2021, 2 stations are monitored every year (Miljøstyrelsen et al., 2017).

Blue mussels (*Mytilus edulis*)

In blue mussels (*Mytilus edulis*), one indicator measured as part of the Danish monitoring programme is "lysosomal membrane stability" (LMS) (OSPAR pre-CEMP: "*general biological effect*"). This indicator was also part of the HELCOM pre-core indicator list, but it was not anticipated to be ready for use in HOLAS II (HELCOM HOD, 2016).

LMS is one type of the lysosomal responses that are used as effect biomarkers indicative of the general stress triggered by pollution in the marine environment (Davies & Vethaak, 2012). LMS can be affected when certain physiological or pathological conditions occur, including those that can be induced by pollutants (Martínez-Gómez et al., 2015). The ACs from OSPAR pre-CEMP category listed are in Table 5.

Table 5. Assessment criteria for biological effect indicators used in blue mussels, used in the Danish monitoring programme NOVANA (ICES WGBEC, 2012; OSPAR, 2013).

Effect indicator	BAC	EAC
Blue mussels		
Lysosomal membrane stability*	120	50

Notes:

* High numbers indicate better status

In the NOVANA programme, the following components, necessary for monitoring application, are developed or used:

- monitoring guidelines / monitoring methods are in place (Strand & Dahllöf, 2013 based on Moore et al., 2004, revised in Martínez-Gómez et al., 2015; OSPAR, 2007, Annex 6);
- procedure for quality assurance of the method: participation in international workshops with practical exercises (e.g. organized by BEQUALM and OSPAR/ICES (ICES/OSPAR, 2010)). Participation in Nordic workshops in 2013 and 2019;
- procedure for quality control of monitoring data by the Marine Topic Center, Aarhus University (Høgslund et al., 2019);
- assessment criteria – Table 5;
- monitoring databases – national database MFS-base (data from MFS-base will be transferred to a new monitoring database at the Danish EPA); international database – ICES DOME;
- temporal and spatial scope – included in NOVANA programme since 2004: within the period of 2017-2021, 2 stations are monitored every year (Miljøstyrelsen et al., 2017).

Marine gastropods

"Imposex and Intersex in marine gastropods" (OSPAR CEMP: "*Organotins concentrations and biological effects*"; HELCOM Core indicator: "*Tributyltin (TBT) and imposex*") are biological effects indicating exposure to and effects of organotin compounds, primarily TBT.

Imposex and intersex are phenomena of masculinisation of females, but they differ morphologically. Imposex in female gastropods primarily entails a development of a pseudo-penis and/or vas deferens, while intersex is malformations of the female reproductive organs or a complete conversion of the female reproductive organs to male organs (Bergkvist & Magnusson, 2016).

For biological effect measurements, the indices used are Vas Deferens Sequence Index (VDSI) and imposex stage (IMPS) for imposex and the Intersex Sequence Index (ISI) and intersex stage (INTS) for intersex.

In Denmark, imposex and intersex in five species of gastropods have been reported as part of the NOVANA programme (Table 6). However, because intersex in the periwinkle *Littorina littorea* is not as sensitive as the imposex indicators, it is only relevant for use in highly TBT polluted harbor areas above the EAC-value for the other more sensitive gastropod species.

Table 6. *Assessment criteria for marine gastropods, biological effect – imposex that during the years have been applied in the NOVANA programme* (OSPAR QSR 2023, HELCOM, 2017).

Marine gastropod	Index	BAC	EAC
Neptunea antiqua	VDSI	0.3	2
Nucella lapillus	VDSI	0.3	2
Hinia reticulata	VDSI	0.1	0.3
Buccinum undatum	VDSI	0.1	0.3
Littorina littorea	ISI	-	0.1*

Notes:

* Adopting value below 0.3

Earlier, OSPAR ACs also reported for five species of gastropods (*Nassarius reticulatus*, *Buccinum undatum*, *Neptunea antique*, *Littorina littorea*). By using data on imposex/intersex in sympatric populations of affected gastropods of different species, the assessment criteria were extended to a range of species used. However, in the latest version of OSPAR CEMP (extracted on August 19th, 2021) only four species, i.e. without *Littorina littorea*, are currently listed.

On the other hand, six gastropod species are included as potential monitoring species in the HELCOM description of imposex as a core indicator for the monitoring activities in the Baltic Sea, since the mudsnail (*Peringia ulvae*) is also included as a relevant indicator species. Here, the species-specific assessment criteria for good status (corresponding to EAC-value above) is achieved at the threshold value of VDSI 2 for the sensitive species dog whelk (*Nucella lapillus*) and red whelk (*Neptunea antiqua*). Most other, less sensitive species, are set at good status for a threshold value of VDSI 0.3, with the exception of the mudsnail, for which a good status threshold value is set at only VDSI 0.1

(HELCOM, 2018b). In addition to the use of BAC and EAC-values as the primary assessment criteria, a five class-scheme has also been developed so that comparison of response levels across the different species can be better performed for environmental assessments across the ICES area (Davies & Vethaak, 2012), (Table 7).

Table 7. *OSPAR biological effect assessment criteria for TBT. Assessment criteria for imposex in Nucella lapillus are presented alongside equivalent VDSI/ISI values for sympatric populations of other relevant species* (Davies & Vethaak, 2012).

ASSESSMENT CLASS	*NUCELLA*	*NASSARIUS*	*BUCCINUM*	*NEPTUNEA*	*LITTORINA*
	VDSI	VDSI	VDSI	VDSI	ISI
A (<BAC)	<0.3	<0.3	<0.3	<0.3	<0.3
B (>BAC <EAC)	0.3-<2.0			0.3-<2.0	
C (>EAC)	2.0 <4.0	0.3-2.0	0.3-2.0	2.0-4.0	
D (>EAC)	4.0-5.0	2.0-3.5	2.0-3.5	4.0^	0.3- <0.5
E (>EAC)	>5.0	>3.5	>3.5		0.5-1.2
F (>EAC)					>1.2

In the NOVANA programme, the following components, necessary for monitoring application, are developed or used:

- monitoring guidelines / monitoring methods are in place (Strand, 2013b, based on OSPAR Commission, 2008, Annex 3);
- procedure for quality assurance of the method: regular participation in international laboratory intercalibration exercises and workshops (QUASIMEME);
- procedure for quality control of monitoring data by the Marine Topic Center, Aarhus University (Høgslund et al., 2019);
- assessment criteria – Tables 6-7;
- monitoring database – national database MFS-base (data from MFS-base will be transferred to a new monitoring database at the Danish EPA); international database – ICES DOME;
- temporal and spatial scope – included in NOVANA programme since 1998: within the period of 2017-2021, 14 stations are monitored every second year (Miljøstyrelsen et al., 2017).

Reproductive disorders in amphipods (*Gammarus spp.*)

Biological effect measurements in amphipods are reproductive disorders - malformed embryos (HELCOM Supplementary indicator: "*Reproductive disorders: malformed embryos of amphipods*"). This indicator is not included in the OSPAR pre-CEMP list of "General biological effects" (Table S5) and JAMP Guidelines for general biological effects monitoring (OSPAR, 2007).

This indicator is applicable in assessment units shared by Finland and Sweden. In Denmark, development of this indicator is initiated by DCE and currently being developed in a pilot study in 2020-2021, financed by the Danish EPA. In Denmark, the "Secondary threshold values" are used as AC for Gammarus spp. (see "Secondary threshold values").

Primary threshold values

The embryo malformation indicator for amphipods is a multimetric indicator based on two variables measured in the sampled population: (1) the proportion of malformed embryos and (2) the proportion of females with more than one malformed embryo (Table 9). Both variables are measured in the same pool of field-collected gravid females. In order to achieve a "good status" for an area under investigation, both variables must be below or equal to their respective threshold values (HELCOM, 2018a).

Table 9. Threshold value for the amphipod *Monoporeia affinis*. BAC and EAC are adopted from Davies & Vethaak, 2012 (HELCOM, 2018a).

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

Secondary threshold values – Gammarids

In Denmark, during the pilot study 2020-21, threshold values for *Gammarus* species are used. In areas where *M. affinis* does not occur naturally or is at low abundances, other amphipods with a similar life cycle and reproduction biology can be used. In the Gulf of Finland and the Gdansk Bay, secondary thresholds were established for gammaridean amphipod species (Table 10). 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 (HELCOM, 2018a).

Table 10. Secondary thresholds for the gammaridean amphipods *Gmelinoides fasciatus*, *Pontogammarus robustoides* and *Gammarus tigrinus* (based on Gulf of Finland monitoring data, Russia) (HELCOM, 2018a).

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

In Denmark, the following components, necessary for monitoring application, are used:

- monitoring guidelines / monitoring methods reported in HELCOM and ICES TIMES are used in a pilot project 2020-2021 (HELCOM, 2018a; Sundelin et al., 2008);
- quality assurance procedures: internal quality assessment, method comparison in meeting within HELCOM EN HZ working group, participation in the international BEAST workshop 2012;
- quality control of monitoring is not in place;
- assessment criteria – Tables 9,10;
- monitoring database – national HSD database is under development;
- temporal and spatial scope – currently a pilot study has been conducted in the period 2020-2021, 2-3 stations each year.

A summary table for all indicators used in the Danish monitoring programme NOVANA is presented in Table 11. This summary table recapitulates the detailed information from the chapters above, using the list of coordination tools, which, according to CEMP, should be in place in order to be applicable as functional indicators in environmental assessment.

Table 11. Summary table for all indicators utilised in the Danish monitoring programme. The table is based on the list of coordination tools that should be in place according to coordinated monitoring and assessment of a component of the CEMP (OSPAR CEMP 2020; NOVANA 2017).

Coordination tools	CYP1A enzymatic activity (EROD), Eelpout	PAH-metabolites in bile, Eelpout	Reproductive success in eelpout	Lysosomal membrane stability" (LMS), Blue mussels	Imposex and Intersex in marine gastropods	Reproductive disorders: malformed embryos of amphipods
Monitoring and/or data collection guidelines	In place (Strand, 2013a)	In place (Strand, 2013a)	In place (Strand, 2013a)	In place (Strand & Dahllöf, 2013)	In place (Strand, 2013b)	Pilot project, 2020-2021
Coordinated arrangements for data submission and management (databases);	MFS-base/ODA; ICES DOME	MFS-base/ODA; ICES DOME	MFS-base/ODA; ICES DOME	MFS-base/ODA; ICES DOME	MFS-base/ODA; ICES DOME	Pilot project, 2020-2022
Quality assurance tools	Förlin, 2012; BEQUALM; Høgslund et al., 2019	Förlin, 2012; BEQUALM; Kammann et al., 2013; Høgslund et al., 2019	Förlin, 2012; Høgslund et al., 2019	BEQUALM; OSPAR/ICES workshop; Høgslund et al., 2019	QUASIMEME, Høgslund et al., 2019	Pilot project, 2020-2023
Assessment criteria (AC) and, where relevant, procedures for aggregation or integration of data prior to assessment	OSPAR pre-CEMP;	OSPAR pre-CEMP; HELCOM	ICES WGBEC 2012; OSPAR 2013	OSPAR pre-CEMP; ICES WGBEC 2012	OSPAR CEMP HELCOM CORE	ACs developed in HELCOM will be evaluated.
Temporal and spatial scope	1-2 stations, frequency 2017-21: 5/5.	1-2 stations, frequency 2017-21: 5/5.	1-2 stations, frequency 2017-21: 5/5.	2 stations, frequency 2017-21: 5/5.	14 stations, frequency 2017-21: 3/5.	2-3 station per year during pilot project.

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QUASIMEME, Quality Assurance of Information for Marine Environmental Monitoring In Europe. About Wepal / Quasimeme - WEPAL

Supplementary tables

Table S1. Assessment criteria from Report of the Working Group on the Biological Effects of Contaminants (WGBEC), 2013. "*Current Assessment criteria for biological effects measurements. Values are given for both background assessment levels (BAC) and environmental assessment criteria (EAC), as available. Values in bold have been updated by WGBEC in 2012 or 2013, changes made in 2013 are described below the table*" (ICES WGBEC, 2013).

Biological Effect	Applicable to:	BAC	EAC
VTG in plasma; µg/ml	Cod	0.23	
	Flounder	0.13	
Reproductive success in fish	Eelpout, Zoarces viviparus		
Mean prevalence (%) of:	Malformed fry	1	2
	Late dead fry	2	4
	Early dead fry	2.5	5
	Total abnormal fry	5	10
EROD; pmol/mg protein pmol/min/ mg protein S9 * pmol/min/ mg microsomal protein	Dab (F)	178	
	Dab (M)	147	
	Dab (M/F)	680*	
	Flounder (M)	24	
	Plaice (M)	9.5	
	Cod (M/F)	145*	
	Plaice (M/F)	255*	
	Four spotted megrim (M/F)	13*	
	Dragonet (M/F)	202*	
	Red mullet (M)- April	208	
	Red mullet (M/F)- October 12-18 cm; GSI<1 Bottom temperature 16-20ºC	115*	
	Eelpout (F)	10	

Table S1. *Continued.*

Biological Effect	Applicable to:	BAC	EAC
PAHs Bile metabolites; (1) ng/ml; HPLC-F (2) pyrene-type µg/ml; synchronous scan fluorescence 341/383 nm (3) ng/g GC/MS * 1-OH pyrene ** 1-OH phenanthrene	Dab	16 (1) * 3.7 (1) ** 0.15 (2)	 22(2)
	Cod	21 (1) * 2.7 (1) ** 1.1 (2)	483 (3) * 528 (3) ** 35 (2)
	Flounder	16 (1) * 3.7 (1) ** 1.3 (2)	 29(2)
	Haddock	13 (1) * 0.8 (1) ** 1.9 (2)	 35(2)
	Eelpout	92 (1) * 7.9 (1) **	
	Herring	143(1) * 2.6(1) **	
DR-Luc; ng TEQ/kg dry wt, silica clean up	Sediment (extracts)	10	40
DNA adducts; nm adducts mol DNA	Dab	1	4,0
	Flounder	1	4,0
	Long Rough Dab		4,0
	Halibut		5,8
	Herring and sprat		0,39
	Cod	1.6	6,7
	Haddock	3.0	6,7
Bioassays; % mortality	Sediment, Corophium	20	60
	Sediment, Arenicola	10	50
	Water, copepod	10	50
Bioassays; % abnormality	Water, oyster embryo	20	50
	Water, mussel embryo	30	50
	Water, sea urchin embryo	10	50
Bioassay; % growth	Water, sea urchin embryo	30	50
Lysosomal stability; minutes	Cytochemical; liver all species	20	10
	Neutral Red Retention: all species	120	50
Micronuclei; 0/00 (frequency of micronucleated cells) 1 Gill cells 2 Haemocytes 3 Erythrocytes	Mytilus edulis	2.5 1 2.5 2	
	Mytilus galloprovincialis	3.9 2	
	Mytilus trossulus	4.5 2	
	Flounder	0.3 3	
	Dab	0.5 3	
	Eelpout	0.4 3	

Table S1. *Continued.*

Biological Effect	Applicable to:	BAC	EAC
	Cod	0.4 3	
	Red mullet (M/F) 12-18 cm; GSI<1 Bottom temperature 16-20ºC Autumn (October)	0.4 3	
Comet Assay; % DNA Tail	Mytilus edulis	10	
	Dab	5	
	Cod	5	
Stress on Stress; days	Mytilus sp.	10	5
AChE activity; nmol.min-1 mg prot-1 1 gills 2 muscle tissue 3 brain tissue * French Atlantic waters ** Portuguese Atlantic waters + French Mediterranean Waters ++ Spanish Mediterranean Waters +++ Baltic sea	Mytilus edulis	30 1*	21 1*
		26 1**	19 1**
	Mytilus galloprovincialis	291+	201+
		15 1++	10 1++
	Flounder	235 2*	165 2*
	Dab	150 2*	105 2*
	Red mullet	155 2+	109 2+
	Red mullet (M/F) 12-18 cm; GSI<1 Bottom temperature 15-16ºC Autumn (October)	1183++	833++
	Eelpout	124 2+++	87 2+++
Externally visible diseases*** Ep,Ly,Ul Ep,Ly,Ul Ac,Ep,Fi,Hp,Le,Ly,St,Ul,Xc Ac,Ep,Fi,Hp,Le,Ly,St,Ul,Xc Ac,Ep,Hp,Le,Ly,St,Ul,Xc Ac,Ep,Hp,Le,Ly,St,Ul,Xc Italics: ungraded, bold: graded NA: Not applied	Dab	Fish Disease Index (FDI): F: 1.32, 0.216 M: 0.96, 0.232 F: 1.03, 0.349 M: 1.17, 0.342 F: 1.09, 0.414 M: 1.18, 0.398 M: males F: females	Fish Disease Index (FDI): F: NA, 54.0 M: NA, 47.7 F: 50.6, 19.2 M: 38.8, 16.1 F: 48.3, 21.9 M: 35.2, 16.5

Table S1. *Continued.*

Biological Effect	Applicable to:	BAC	EAC
Liver histopathology-non specific	Dab	NA	Statistically significant increase in mean FDI level in the assessment period compared to a prior observation period or Statistically significant upward trend in mean FDI level in the assessment period
Liver histopathology-contaminant-specific	Dab	Mean FDI <2	Mean FDI ≥ 2 A value of FDI = 2 is, e. g., reached if the prevalence of liver tumours is 2 % (e. g., one specimen out of a sample of 50 specimens is affected by a liver tumour). Levels of FDI ≥ 2 can be reached if more fish are affected or if combinations of other toxicopathic lesions occur.
Macroscopic liver neoplasms	Dab	Mean FDI <2	Mean FDI ≥ 2 A value of FDI = 2 is reached if the prevalence of liver tumours (benign or malignant) is 2 % (e. g., one specimen out of a sample of 50 specimens is affected by a liver tumour). If more fish are affected, the value is FDI > 2.

Table S1. *Continued.*

Biological Effect	Applicable to:	BAC	EAC
Intersex in fish; % prevalence	Dab Flounder Cod Red mullet Eelpout	5	
Scope for growth Joules/hr/g dry wt.	Mussel (Mytilus sp.) (provisional, further validation required)	25	15
Hepatic metallothionein ìg/g (w.w.) 1 Whole animal 2Digestive gland 3Gills * Differential pulse polarography	Mussel edulis	0.6 1* 2.0 2* 0.6 3*	
	Mytilus galloprovincialis	2.0 1* 3.92* 0.6 3*	
Histopathology in mussels	VVbas: Cell type composition of digestive gland epithelium; µm3/µm3 (quantitative)	0.12	0.18
	MLR/MET: Digestive tubule epithelial atrophy and thinning; µm/µm (quantitative)	0.7	1.6
	VVLYS and Lysosomal enlargement; µm3/µm3 (quantitative)	VvLYS 0.0002	V>0.0004
	S/VLYS: µm2/µm3	4	
	Digestive tubule epithelial atrophy and thinning (semi-quantitative)	STAGE ≤1	STAGE 4
	Inflammation (semi-quantitative)	STAGE ≤1	STAGE 3
Imposex/intersex in snails VDSI	Nucella lapillus	<0.3	<2

*****: Assessment criteria for the assessment of the Fish Disease Index (FDI) for externally visible diseases in common dab (*Limanda limanda*). Abbreviations used: Ac, *Acanthochondria cornuta;* Ep, Epidermal hyperplasia/papilloma; Fi, Acute/healing fin rot/erosion; Hp, Hyperpigmentation; Le, *Lepeophtheirus sp.;* Ly, Lymphocystis; St, *Stephanostomum baccatum;* Ul, Acute/healing skin ulcerations; Xc, X-cell gill disease.**

Full details of how the original assessment criteria and how they were derived can be found in the SGIMC 2010 and SGIMC 2011 and WKIMON 2009 reports on the ICES website and in the OSPAR Background Documents for individual biological effects methods. In addition, amendments and the justification for same can be found in the ICES WGBEC report 2012.

Table S2. *Assessment criteria (OSPAR pre-CEMP) for CYP1A enzymatic activity (EROD) in fish liver, measured using two different liver tissue fractions (see notes below the table).* (OSPAR pre-CEMP, extracted on August 19th, 2021).

species	common name	sex	matrix	BAC
Gadus morhua	cod	both	liver microsome	145.0
Limanda limanda	dab	female	liver S9	178.0
		male	liver S9	147.0
		both	liver microsome	680.0
Callionymus lyra	dragonet	both	liver microsome	202.0
Platichthys flesus	flounder	male	liver S9	24.0
Lepidorhombus boscii	four spotted megrim	both	liver microsome	13.0
Pleuronectes platessa	plaice	male	liver S9	9.5
		both	liver microsome	255.0
Mullus barbatus	red mullet	male	liver S9	208.0

Notes:
units are pmol min^{-1} mg S9 $protein^{-1}$ or pmol min^{-1} mg microsomal protein−1 for the liver S9 and liver microsome matrices respectively;
there are no EACs;
low values indicate healthy organisms.

Table S3. *Assessment criteria (OSPAR pre-CEMP) for biliary PAH metabolites in fish, measured using three different analytical techniques (see notes below the table).* (OSPAR pre-CEMP, extracted on August 19th, 2021).

bile metabolite	species	common name	method	BAC	EAC
1-OH pyrene	*Gadus morhua*	cod	HPLC-F	21.00	
			GC-MS		483
	Limanda limanda	dab	HPLC-F	16.00	
	Platichthys flesus	flounder	HPLC-F	16.00	
	Melanogrammus aeglefinus	haddock	HPLC-F	13.00	
1-OH pyrene equivalents	*Gadus morhua*	cod	SSF	1.10	35
	Limanda limanda	dab	SSF	0.15	22
	Platichthys flesus	flounder	SSF	1.30	29
	Melanogrammus aeglefinus	haddock	SSF	1.90	35
1-OH phenanthrene	*Gadus morhua*	cod	HPLC-F	2.70	
			GC-MS		528
	Limanda limanda	dab	HPLC-F	3.70	
	Platichthys flesus	flounder	HPLC-F	3.70	
	Melanogrammus aeglefinus	haddock	HPLC-F	0.80	

Notes:
HPLC-F is high performance liquid chromatography - fluorescence, GC-MS is gas chromatography - mass spectrometry, and SSF is synchronous scan fluorescence 341/383 nm;
units are ng ml^{-1} for HPLC-F, ng g^{-1} for GC-MS, and pyrene-type µg ml^{-1} for SSF;
the proliferation of methods and units are a mystery to all those who have not devoted their lives to the study of bile metabolites;
low values indicate healthy organisms.

Table S4. PAH and metal-specific relevant biological Effects. (OSPAR pre-CEMP, extracted on August 19th, 2021)		
Field	Description	Validated Entry
[Table title]	Name of monitoring programme.	PAH and metal-specific relevant Biological Effects
[ProgrammeDescription]	Description of the monitoring programme.	The PAH and metal-specific relevant Biological Effects monitoring is carried out within OSPAR's pre-Coordinated Environmental Monitoring Programme (CEMP) (Agreement 2016-01). Pre-CEMP monitoring work on hazardous substances comprises monitoring and assessment of the sources and pathways of contaminants and their concentrations and effects in the marine environment. OSPAR has agreed within the context of the pre-CEMP that certain components for hazardous substances are not monitored on a mandatory basis. Pre-CEMP component H-10 sets out monitoring of PAH and metal-specific biological effects.
[OtherPoliciesConventions]	Monitoring for other Union legislation or international agreements that contributes to the programme.	OSP-CEMP
[Contracting Parties monitoring]	Which other countries are involved in practical implementation of this monitoring programme, and what is the degree of cooperation.	Coordinated data collection BE, DE, DK, FR, NL, NO, SE, UK
[Contracting Parties supplying data]		BE, DE, DK, FR, NL, NO, SE, UK
[Contracting Parties with an "opt out"]		
[Temporal scope]	Start (and end) date of the programme.	1989 - 9999
[Spatial scope]	Spatial coverage of the programme according to the jurisdictional zones of marine waters.	EEZ (or similar, e.g. Contiguous Zone, Fishing Zone, Ecological Protection Zone)

[Aggregation of Data]	At which scale can the data from the sub-programme be aggregated for environmental assessments?	Sub-region
[MarineReportingUnit]	Area(s) where the programme takes place.	marineSubregion marineSubdivision
[Monitoring Purpose]	Purpose of the programme aimed at collecting data and information.	Pressures in the marine environment
[MonitoringType]	Type of monitoring (in-situ, remote sensing, etc.).	In-situ sampling offshore
[Indicator Metric]	Feature(s) monitored (ecosystem components, pressures, activities).	PresEnvContNonUPBTs PresEnvContUPBTs
[Elements]	Element(s) monitored (e.g. species, habitats, contaminants).	Total PAHs (Benzo(a)pyrene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(ghi)perylene, Indeno(1,2,3-cd)pyrene)
[Element Monitored]		
[Parameter Measured]	Parameter(s) monitored.	CONC-B CONC-S
[Monitoring Guidelines/Monitoring Method in place]	Guidelines/protocols describing the method for monitoring.	OTH OSP-026
[MonitoringMethodOther]	Guidelines/protocols describing the method for monitoring.	CEMP guidelines for coordinated monitoring for hazardous substances (Agreement 2016-04)
[Quality Assurance Procedures in Place]	In addition to a specified method, is there any additional Quality Assurance used?	Currently developed by the ICES WGBEC CEFAS laboratory proficiency testing scheme: BEQUALM
[Quality Control]	What type of Quality Control is used?	Delayed mode
[Data submission Frequency (and deadline)]	Frequency of the monitoring	Yearly
[Applicable MSFD GES Criteria]	Indicator(s) to which the programme contributes.	D8C1

[Data Depository]	Link to where monitoring data can be accessed (Art. 19(3))	https://ocean.ices.dk/OHAT/
[Data Custodian]		ICES Datacentre
[Assessment Guidelines]		https://ocean.ices.dk/ohat/trDocuments/2019/help_ac_biota_biological_effects.html https://ocean.ices.dk/ohat/trDocuments/2019/help_methods_biota_biological_effects.html
[Assessment tools available]		Background Reference Concentrations; Ecotoxicological Assessment Criteria (Davies & Vetaack, 2012)
[Nature of data]		Processed

Table S5. General biological effects (Whole sediment bioassays, Sediment Pore-Water and Elutriate Bioassays, Water Bioassays, CYP1a, Lysosomal Stability, Liver Histopathology/ Macroscopic liver neoplasms, Externally Visible Fish Diseases, Reproductive Success in Fish). (OSPAR pre-CEMP, extracted on August 19th, 2021).

Field	Description	Validated Entry
[Table title]	Name of monitoring programme.	General Biological Effects (Whole sediment bioassays, Sediment Pore-Water and Elutriate Bioassays, Water Bioassays,CYP1a, Lysosomal Stability, Liver Histopathology/ Macroscopic liver neoplasms, Externally Visible Fish Diseases, Reproductive Success in Fish)
[ProgrammeDescription]	Description of the monitoring programme.	The General Biological Effects (Whole sediment bioassays, Sediment Pore-Water and Elutriate Bioassays, Water Bioassays,CYP1a, Lysosomal Stability, Liver Histopathology/ Macroscopic liver neoplasms, Externally Visible Fish Diseases, Reproductive Success in Fish)monitoring is carried out within OSPAR's pre-Coordinated Environmental Monitoring Programme (CEMP) (Agreement 2016-01). Pre-CEMP monitoring work on hazardous substances comprises monitoring and assessment of the sources and pathways of contaminants and their concentrations and effects in the marine environment. OSPAR has agreed within the context of the pre-CEMP that certain components for hazardous substances are not monitored on a mandatory basis. Pre-CEMP component H-11 sets out monitoring of general biological effects.
[OtherPoliciesConventions]	Monitoring for other Union legislation or international agreements that contributes to the programme.	OSP-CEMP
[Contracting Parties monitoring]	Which other countries are involved in practical implementation of this monitoring programme, and what is the degree of cooperation.	Coordinated data collection BE, DE, DK, FR, NL, NO, SE, UK
[Contracting Parties supplying data]		BE, DE, DK, FR, NL, NO, SE, UK
[Contracting Parties with an "opt out"]		
[Temporal scope]	Start (and end) date of the programme.	2010 - 9999
[Spatial scope]	Spatial coverage of the programme according to the jurisdictional zones of marine waters.	EEZ (or similar, e.g. Contiguous Zone, Fishing Zone, Ecological Protection Zone)
[Aggregation of Data]	At which scale can the data from the sub-programme be	Sub-region

	aggregated for environmental assessments?	
[MarineReportingUnit]	Area(s) where the programme takes place.	marineSubregion marineSubdivision
[Monitoring Purpose]	Purpose of the programme aimed at collecting data and information.	Environmental state and impacts Pressures in the marine environment
[MonitoringType]	Type of monitoring (in-situ, remote sensing, etc.).	In-situ sampling offshore
[Indicator Metric]	Feature(s) monitored (ecosystem components, pressures, activities).	PresEnvContNonUPBTs PresEnvContUPBTs
[Elements]	Element(s) monitored (e.g. species, habitats, contaminants).	All Contaminants
[Element Monitored]		Effects of contaminants
[Parameter Measured]	Parameter(s) monitored.	CONC-B CONC-S CONC-W
[Monitoring Guidelines/Monitoring Method in place]	Guidelines/protocols describing the method for monitoring.	OTH OSP-025
[MonitoringMethodOther]	Guidelines/protocols describing the method for monitoring.	CEMP guidelines for coordinated monitoring for hazardous substances (Agreement 2016-04)
[Quality Assurance Procedures in Place]	In addition to a specified method, is there any additional Quality Assurance used?	ICES WGBEC RingTests
[Quality Control]	What type of Quality Control is used?	Delayed
[Data submission Frequency (and deadline)]	Frequency of the monitoring	Yearly
[Applicable MSFD GES Criteria]	Indicator(s) to which the programme contributes.	D8C1

[Data Depository]	Link to where monitoring data can be accessed (Art. 19(3))	https://ocean.ices.dk/OHAT/
[Data Custodian]		ICES Datacentre
[Assessment Guidelines]		https://ocean.ices.dk/oat/trDocuments/2019/help_ac_biota_biological_effects.html https://ocean.ices.dk/oat/trDocuments/2019/help_methods_biota_biological_effects.html
[Assessment tools available]		Background Reference Concentrations; Ecotoxicological Assessment Criteria
[Nature of data]		Processed

Table S6. Organotins concentrations and biological effects. (OSPAR CEMP, extracted on August 19th, 2021).		
Field	Description	Validated Entry
[Table title]	Name of monitoring programme.	Organotins concentrations and biological effects
[ProgrammeDescription]	Description of the monitoring programme.	The organotins concentrations and biological effects concentrations monitoring is carried out within OSPAR's Coordinated Environmental Monitoring Programme (CEMP) (Agreement 2016-01). CEMP monitoring work on hazardous substances comprises monitoring and assessment of the sources and pathways of contaminants and their concentrations and effects in the marine environment. OSPAR has agreed within the context of the CEMP that certain components for hazardous substances are to be monitored on a mandatory basis, subject to clearly defined and agreed conditions. CEMP component H-4 sets out monitoring of tributyl tin (TBT)-specific biological effects and TBT in sediment or biota (Appendix H4). Monitoring of TBT concentrations in the marine environment in either sediments or biota should be carried out in parallel with monitoring of TBT-specific biological effects.
[OtherPoliciesConventions]	Monitoring for other Union legislation or international agreements that contributes to the programme.	OSP-CEMP
[Contracting Parties monitoring]	Which other countries are involved in practical implementation of this monitoring programme, and what is the degree of cooperation.	Coordinated data collection BE, DE, DK, ES, IE, IS, NL, NO, PT, SE, UK
[Contracting Parties supplying data]		BE, DE, DK, ES, IE, IS, NL, NO, PT, SE, UK
[Contracting Parties with an "opt out"]		
[Temporal scope]	Start (and end) date of the programme.	1978 - 9999
[Spatial scope]	Spatial coverage of the programme according to the jurisdictional zones of marine waters.	Coastal waters (WFD)
[Aggregation of Data]	At which scale can the data from the sub-programme be aggregated for environmental assessments?	Sub-region
[MarineReportingUnit]	Area(s) where the programme takes place.	marineSubregion marineSubdivision
[Monitoring Purpose]	Purpose of the programme aimed at collecting data and information.	Environmental state and impacts Pressures in the marine environment

[MonitoringType]	Type of monitoring (in-situ, remote sensing, etc.).	In-situ sampling offshore
[Indicator Metric]	Feature(s) monitored (ecosystem components, pressures, activities).	PresEnvContNonUPBTs PresEnvContUPBTs
[Elements]	Element(s) monitored (e.g. species, habitats, contaminants).	Tributyltin compounds
[Element Monitored]		TBT-specific biological effects: Vas Deferens Sequence, Imposex Stage, Intersex Stage Organotins in Sediments: Mono & Dibutyltin; Mono, Di & Triphenyltin; Tributyltin Organotins in Biota: Mono & Dibutyltin; Mono, Di & Triphenyltin; Tributyltin
[Parameter Measured]	Parameter(s) monitored.	CONC-B CONC-S
[Monitoring Guidelines/Monitoring Method in place]	Guidelines/protocols describing the method for monitoring.	OTH OSP-001 OSP-018 OSP-025 OSP-026 OSP-028
[MonitoringMethodOther]	Guidelines/protocols describing the method for monitoring.	CEMP guidelines for coordinated monitoring for hazardous substances (Agreement 2016-04)
[Quality Assurance Procedures in Place]	In addition to a specified method, is there any additional Quality Assurance used?	External laboratory proficiency testing scheme: QUASIMEME
[Quality Control]	What type of Quality Control is used?	Delayed mode
[Data submission Frequency (and deadline)]	Frequency of the monitoring	Yearly
[Applicable MSFD GES Criteria]	Indicator(s) to which the programme contributes.	D8C1 D8C2
[Data Depository]	Link to where monitoring data can be accessed (Art. 19(3))	https://ocean.ices.dk/OHAT/
[Data Custodian]		ICES Datacentre

[Assessment Guidelines]	https://ocean.ices.dk/oat/trDocuments/2019/help_ac_sediment_organo-metals.html https://ocean.ices.dk/oat/trDocuments/2019/help_methods_sediment_organo-metals.html https://ocean.ices.dk/oat/trDocuments/2019/help_ac_biota_organo-metals.html https://ocean.ices.dk/oat/trDocuments/2019/help_methods_biota_organo-metals.html
[Assessment tools available]	https://ocean.ices.dk/OHAT/
[Nature of data]	Processed