

Integrated chemical and biological assessment of contaminant impacts in selected European coastal and offshore marine areas

Ketil Hylland<sup>1</sup>, Craig D. Robinson<sup>2</sup>, Thierry Burgeot<sup>3</sup>, Concepción Martínez-Gómez<sup>4</sup>, Thomas Lang<sup>5</sup>, Jörundur Svavarsson<sup>6</sup>, John E. Thain<sup>7</sup>, A. Dick Vethaak<sup>8</sup>, Matthew J. Gubbins<sup>2</sup>

<sup>1</sup> *Department of Biosciences, University of Oslo, PO Box 1066, Blindern, N-0316 Oslo, Norway*

<sup>2</sup> *Marine Scotland Science, Marine Laboratory, 375 Victoria Road, Aberdeen, AB11 9DB, UK*

<sup>3</sup> *IFREMER, Laboratory of Ecotoxicology, Rue de l'Île d'Yeu, B.P. 21105, 44311 Nantes Cédex 03, France*

<sup>4</sup> *Instituto Español de Oceanografía (IEO), Oceanographic Centre of Murcia, Varadero 1, PO BOX 22, 30740 San Pedro del Pinatar (Murcia), Spain.*

<sup>5</sup> *Thünen Institute of Fisheries Ecology, Deichstr. 12, 27472 Cuxhaven, Germany*

<sup>6</sup> *University of Iceland, Askja – Natural Science Building, Sturlugata 7, 101 Reykjavík, Iceland*

<sup>7</sup> *Cefas Weymouth Laboratory, Barrack Road, The Nothe, Weymouth, Dorset, DT4 8UB, UK*

<sup>8</sup> *Deltares, Marine and Coastal Systems, P.O. Box 177, 2600 MH Delft, The Netherlands / VU University Amsterdam, Amsterdam Global Change Institute, Institute for Environmental Studies, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands*

Communicating author: Ketil Hylland, ketilhy@ibv.uio.no, phone +4722857315/+4741451694

1 **Abstract**

2

3 This paper reports a full assessment of results from ICON, an international workshop on  
4 marine integrated contaminant monitoring, encompassing different matrices (sediment,  
5 fish, mussels, gastropods), areas (Iceland, North Sea, Baltic, Wadden Sea, Seine estuary  
6 and the western Mediterranean) and endpoints (chemical analyses, biological effects).  
7 ICON has demonstrated the use of a framework for integrated contaminant assessment  
8 on European coastal and offshore areas. The assessment showed that chemical  
9 contamination did not always correspond with biological effects, indicating that both are  
10 required. The framework can be used to develop assessments for EU directives. If a  
11 95% target were to be used as a regional indicator of MSFD GES, Iceland and offshore  
12 North Sea would achieve the target using the ICON dataset, but inshore North Sea, Baltic  
13 and Spanish Mediterranean regions would fail.

14

15

16

17

18

19 **Keywords:** ICON, contaminants, European seas, biological effects, assessment

20

## 21 **Introduction**

22 Thousands of tonnes of waste are released into European seas every minute, containing  
23 chemicals that have the potential to accumulate in marine organisms and/or affect their  
24 health. As discussed in Borja et al. (2010), it is crucial in this context to have a clear  
25 understanding of how it can be determined whether organisms or populations in an  
26 area are affected by pollution and if so, the extent to which they are impacted. With  
27 regards to chemicals, this implies quantifying chemical-specific effects on marine  
28 organisms or processes. In addition to a required knowledge of effects, there are reasons  
29 why it may also useful to have information about concentrations of chemicals in  
30 organisms or abiotic matrices: (i) to link observed effects to specific chemicals for  
31 regulatory purposes, (ii) to ensure concentrations are not above limits set for human  
32 consumption, and finally (iii) to document the presence of chemicals that may or may  
33 not cause effects. As support for effects, it is the exposure of organisms to chemicals that  
34 matters. For persistent bioaccumulating substances, exposure can be estimated through  
35 measuring the concentration of chemicals or their metabolites in the tissues of the target  
36 organism (e.g. Hylland et al., 2009) or in other matrices such as passive samplers (Utvik  
37 & Gärtner, 2006), sediments or non-target organisms in the same habitat, e.g. blue  
38 mussels. Some polluting chemicals may however be quickly degraded or present at  
39 concentrations below the detection limit of routine chemical analyses, but still cause  
40 impacts, e.g. many endocrine disrupting substances, organophosphate pesticides and  
41 pharmaceuticals. In this case, biological responses will be the most sensitive method by  
42 which to detect their presence, e.g. through the inhibition of acetylcholinesterase as a  
43 result of organophosphate exposure (Bocquené et al., 1993) or increased plasma  
44 concentrations of vitellogenin in juvenile fish as a result of oestrogen exposure (Allen et  
45 al., 1999). To understand the possible environmental consequences and regulate inputs  
46 of contaminating chemicals, we therefore need to know both the concentrations of  
47 contaminants in appropriate matrices as well as how they affect organisms. The two  
48 types of measurements, chemical and biological, should ideally be combined in an  
49 integrated assessment (cf. Davies & Vethaak, 2012). Any monitoring programme  
50 underpinning such an assessment will however produce a very extensive and complex  
51 data matrix, which will require some sort of aggregation procedure prior to being used  
52 for regulatory decisions. Such aggregation procedures are generally termed "indicators",  
53 see e.g. Rees et al. (2008). Indicators have previously been developed separately to

54 aggregate or combine chemical analyses (see e.g. OSPAR, 2010) or biological responses,  
55 e.g. the health assessment index, HAI (Adams et al., 1993), biological assessment index,  
56 BAI (Broeg et al., 2005), an expert system (Viarengo et al., 2000; Dagnino et al., 2007),  
57 the integrated biological response, IBR (Devin et al., 2014), the biomarker response  
58 index (BRI) (Hagger et al., 2008) or the integrative biomarker Index, IBI (Marigómez et  
59 al., 2013). In addition, there are some practical examples of integrating or combining  
60 chemical analyses and biological responses, such as in the UK Fullmonti project,  
61 including chemical analyses, benthic community status and fish health (described in  
62 Thain et al., 2008) or by using a weight-of-evidence approach (see e.g. Chapman et al.,  
63 2002). In some national programmes, the interpretation of fish health is aided by taking  
64 account of contaminant levels in addition to confounding factors such as size and  
65 gender, and environmental factors such as temperature and season (see e.g. Sandström  
66 et al., 2005; Hylland et al., 2008, 2009; Vethaak et al., 2008). The main difference  
67 between the framework used here (described in Vethaak et al., this issue-a) and other  
68 indices is that the current framework is based on internationally agreed threshold  
69 criteria for biological responses and tissue residues of chemicals, identifying responses  
70 above background, responses that indicate probable impacts at the population level and  
71 concentration of chemicals above thresholds (see Robinson et al., this issue). In addition,  
72 the framework includes more matrices than most other indices and is flexible in the  
73 species included, as long as criteria exist for core methods.

74  
75 Over the last decade, Europe has implemented two directives that largely direct the  
76 management of the environmental conditions of coastal and offshore marine areas, the  
77 Water Framework Directive (WFD, 2000/60/EC) and Marine Strategy Framework  
78 Directive (MSFD, 2008/56/EC). Particularly descriptor 8 of MSFD, ‘Concentrations of  
79 contaminants are at levels not giving rise to pollution effects’, is clearly relevant for the  
80 assessment described here for the ICON project (International workshop on marine  
81 integrated contaminant monitoring, see Hylland et al., this issue-a, for a full description).  
82 Using biological responses to provide the information required for descriptor 8 has been  
83 suggested in e.g. Bourlat et al. (2013), Giltrap et al. (2013), Hagger et al. (2008),  
84 Lehtonen et al. (2014) and Lyons et al. (2010). As outlined in Lyons et al. (2010), the  
85 framework described in Vethaak et al. (this issue-a) and applied to the ICON project will

86 output a metric that can be used to determine Good Environmental Practice (GES) in  
87 MSFD.

88

89 The current paper reports on an integrated assessment of the results from the ICON  
90 (International workshop on marine integrated contaminant monitoring) project, using  
91 results reported in Burgeot et al. (this issue), Carney Almroth et al. (this issue), Hylland  
92 et al. (this issue-b), Kammann et al. (this issue), Lang et al. (this issue – a,b), Lyons et al.  
93 (this issue), Martinez-Gomez et al. (this issue –a, b), Robertson et al. (this issue), Vethaak  
94 et al. (this issue-b).

95

96 As described in Vethaak et al. (this issue-a), this indicator of status for each determinant  
97 can then be combined at different levels: matrix, site and region, and expressed with  
98 varying levels of aggregation to graphically represent the proportion of different types  
99 of determinants (or for each determinant, sites within a region) exceeding assessment  
100 criteria. Such an approach has several advantages: (i) the combination of data can be  
101 done for selected levels depending on the type of assessment required and the  
102 monitoring data available, (ii) the representation maintains all the original information  
103 and it is straightforward to identify determinants that exceed the assessment criteria,  
104 (iii) any stage of the assessment can be readily “unpacked” to a previous stage to identify  
105 either contaminant or effects measurements of potential concern or sites contributing to  
106 poor regional assessments (cf. Jennings et al., 2008). In contrast to some other  
107 integrating indicators, e.g. IBI and BRI, there is no weighing of the methods included in  
108 the current framework. The approach is based on the OSPAR regional assessment tool  
109 developed for contaminants (OSPAR, 2010).

110

111

112 **Methods**

113 The assessment criteria used with chemical components of the framework were OSPAR  
114 Background Assessment Criteria (BACs) and Environmental Assessment Criteria (EACs)  
115 or EU Environmental Quality Standards (EQSs); EC food safety regulation limits were  
116 used where EACs or EQSs are not available (OSPAR, 2008). Food safety regulation limits  
117 are not necessarily protective for the environment. Assessment criteria for biological  
118 responses (biomarkers) were from Davies & Vethaak (2012). Initial comparisons (step 1  
119 below) would decide whether the concentration or response for any species or matrix at  
120 any site was less than BAC, between the BAC and EAC, or above EAC. As described in  
121 detail in Hylland et al. (this volume – a) and Vethaak et al. (this volume – a), biological  
122 responses were grouped in either “exposure” or “effect”, subject to whether there is  
123 available data showing adverse effects corresponding to that particular response.

124

125 The sites included in the ICON project are described in Hylland et al. (this issue - a). They  
126 comprised sites from the Mediterranean in the south to Iceland in the north,  
127 encompassing the Seine estuary, Wadden Sea, a range of coastal, estuarine and offshore  
128 sites in the North Sea and one site in the Baltic (Table 1). The two coastal and two  
129 offshore sites on Iceland were included as reference sites.

130

131 The matrices chosen for ICON were sediment, haddock (*Melanogrammus aeglefinus*),  
132 dab (*Limanda limanda*), flounder (*Platichthys flesus*), red mullet (*Mullus barbatus*),  
133 gastropod (*Nucella lapillus*) and mussels (*Mytilus edulis* or *M. galloprovincialis*) (cf.  
134 Hylland et al., this issue-a). The chemical analyses performed in ICON were for PAHs,  
135 PCBs, Cd, Hg and Pb (Robinson et al., this issue). The biological responses included for  
136 fish were (exposure indicators): red blood cell micronucleus frequency, genotoxicity  
137 (comet assay), cytochrome P4501A activity (EROD), bile PAH metabolites (by HPLC),  
138 plasma vitellogenin (VTG) and intersex, and (effect indicators): lysosomal membrane  
139 stability (LMS), acetylcholinesterase inhibition (AChE), bile PAH metabolites (by  
140 synchronous scanning fluorometry, SFF), DNA adduct concentration, external fish  
141 disease, hepatic neoplasms and liver histology. The two methods for PAH metabolite  
142 analyses can be converted one to the other, but only SSF data has been linked directly to  
143 adverse effects in experimental studies, hence the grouping in “exposure” and “effect”.  
144 Effect responses for mussels were acetylcholinesterase inhibition (AChE), stress-on-

145 stress (SoS), scope for growth (SfG), metallothionein (MT), histopathology (histo),  
146 lysosomal membrane stability (LMS), and for gastropods imposex (VDSI). The reader is  
147 referred to Davies & Vethaak (2012) and the relevant chapters of that volume for more  
148 detail on background data and the motivation for selecting methods. The selection of  
149 methods follows on from discussions in the ICES working group on biological effects of  
150 contaminants (WGBEC) over the past two decades (see e.g. ICES, 2010). The original list  
151 of recommended methods were further refined by the ICES/OSPAR working group  
152 SGIMC (ICES, 2011), taking into account additional issues such as cost-benefit and  
153 availability of analytical techniques in different countries. The final selection largely  
154 corresponds to the methods chosen by HELCOM for the Baltic (CORESET) (Lehtonen et  
155 al., 2014). The data from the individual studies in ICON (reported in this special issue)  
156 were compiled and subjected to a five-step procedure, eventually resulting in an overall  
157 assessment of the sites included in ICON. The assessment strategy is transparent and,  
158 depending on the objectives of an assessment, it may be desirable to stop after steps  
159 two, three or four.

160

### 161 **Step 1: Assessment of monitoring data against BAC and EAC**

162 All measurements performed within ICON were compared with the relevant BAC  
163 and EAC for that specific endpoint and species and expressed as a colour depending  
164 on whether the value exceeded the BAC or EAC. Details of calculations can be found  
165 in Davies & Vethaak (2012) and in Vethaak et al. (this volume –a). A red  
166 classification would indicate that the value was above EAC, blue indicated values  
167 below the BAC, while green indicated concentrations or effect responses between  
168 the BAC and EAC. The method for determining whether a response is in either  
169 category can be found in Vethaak et al. (this issue-a). For all biological responses it is  
170 possible to identify a level at which the investigated population would be classified  
171 as being exposed to contaminants, i.e. with values above the background assessment  
172 concentration (BAC), but for only some of the methods will there be data available  
173 that can link the response to e.g. increased mortality in some life stage of the same  
174 species at that concentration, providing the environmental assessment  
175 concentration (EAC).

176

177 **Step 2: Integration of determinants by matrix for a given site**

178 For each of the matrices the results of the individual assessments were aggregated  
179 into three main categories: contaminants, exposure indicators and effects indicators.  
180 For sediment/water, passive sampling and bioassays were done for some sites (see  
181 Vethaak et al., this issue-a). Exposure indicators are biological responses that are not  
182 predictive of "significant" effects, i.e. exceeding EAC, and can hence only be blue or  
183 green. It was found necessary to split the biological effects measurements into two  
184 categories depending on whether an EAC was set for that specific response or not.  
185 Otherwise aggregated information on the proportion of determinants exceeding the  
186 separate AC would be incorrect. For simplicity, these categories have been termed  
187 'exposure indicators' (where an EAC has not been set) and 'effects indicators' where  
188 an EAC (equivalent to significant pollution effect) has been set for the measurement.  
189

190 In future projects with aggregation/integration of the above indicators across  
191 matrices for a specific site, bioassays will be considered 'effects indicators' as EACs  
192 become available. It will be possible to include data from passive sampling and *in*  
193 *vitro* bioassays in both the water and sediment components in the framework  
194 whenever assessment criteria become available.

195  
196 The integration by matrix and category of determinant are expressed by three- or  
197 four-coloured bars showing the proportions of determinants that exceed the BAC  
198 and EAC. To indicate a lack of results for core methods or lack of data, grey has been  
199 used. Each method for contaminant, effect or exposure assessment carries the same  
200 weight, within matrix, in the integration. All determinants carry the same weight in  
201 the assessment as they are perceived to have equivalent significance. That is to say  
202 all determinants either represent a contaminant concentration or effect that is  
203 either above or below background (BAC), or likely to cause (contaminant EAC) or be  
204 indicative of (effect EAC) significant detrimental effects to individuals or  
205 populations of marine organisms.

206



207 **Step 3: Integration of matrices for a site assessment**

208 In order to express the results of assessment for any particular site, assessments  
209 were aggregated across matrices and expressed by determinant category. To  
210 achieve this, results from passive sampling from sediment and water categories  
211 were integrated into the contaminant indicator graphic and bioassays and  
212 gastropod intersex/intersex integrated into 'effects indicators'. Thus the outcome of  
213 assessment of all determinants from all matrices can be expressed for a whole site.  
214 Practically, the process adopted is to sum the percentages of each colour in, say, the  
215 "contaminants" columns for each matrix, and then to scale the sums to a total of  
216 100%.

217  
218 For some assessments, this will be the highest level of aggregation required.  
219 However, for assessments covering larger geographical areas where assessments  
220 need to be undertaken across multiple sites, a further level of integration is required  
221 (steps 4 and 5).

222  
223 For transparency, each determinant group is labelled with the matrices from which  
224 it is comprised. Thus it can quickly be determined whether the site assessment is  
225 comprised of all or just a sub-set of the monitoring matrices.

226

227 **Step 4: Regional assessment across multiple sites**

228 A regional assessment can be done at different levels, i.e. aggregation of data at the  
229 sub-regional, regional and national levels, in different ways to express both the  
230 overall assessment of proportion of determinants (across all matrices) exceeding  
231 both assessment thresholds (BAC/EAC) and by determinant for the region, showing  
232 the proportion of sites assessed in the region that exceed the thresholds. Both  
233 approaches show the overall proportion of determinant/site that exceeds the  
234 threshold for each method.

235

236 **Step 5: Overall assessment**

237 The assessment by region can be aggregated further into a single schematic showing  
238 the proportion all determinants across all sites that exceed BAC and EAC. This can  
239 be used for the purposes of an overall assessment. The overall assessment can be  
240 easily “unpacked” through the steps above to determine which sites and  
241 determinants (effects types or contaminants) are contributing to, for example, the  
242 proportion of red (greater than EAC) data, and thereby potentially leading to failure  
243 to achieve the desired status for a region.

244

245 The assessment criteria for fish were grouped in three categories: concentrations of  
246 selected contaminants, biomarkers of exposure (e.g. PAH metabolites and  
247 cytochrome P4501A (EROD) activity) and biomarkers of effect (e.g. DNA damage,  
248 fish disease). For each category the response at each location was then scored.

249

250 **Results**

251 Assessments were performed by matrix (sediment, mussels, gastropods and fish), by  
252 site and by region.

253

254 **Assessment results by matrix**

255 Contaminant concentrations measured did not exceed EAC values at any of the  
256 offshore sites for sediments, yet at two of these sites (Iceland SE and Firth of Forth  
257 offshore) sediment bioassay results exceeded EAC values, suggesting effects may be  
258 being caused by contaminants not measured in sediment samples (Figure 1). Iceland  
259 SE is adjacent to areas with high volcanic activity, which could result in elevated  
260 concentrations of e.g. metals not analysed for. At inshore sites, concentrations of the  
261 trace metals mercury and lead exceeded EAC values at the Wadden Sea site, the  
262 Baltic Sea site and the Cartagena site in Spain, while mercury also exceeded EAC  
263 values in the Seine estuary and the Firth of Firth, where PAH concentrations also  
264 exceeded EAC. In the Wadden Sea, sediment bioassay results exceeded EACs,  
265 indicating significant effects, presumably resulting from the high trace metal  
266 concentrations recorded.

267

268 The mussel data assessment for Bjarnarhöfn (Iceland) and Palos Cape (SE Spain)  
269 showed good relationship between chemical analytical results and biological  
270 responses, with contaminant concentrations generally below BAC and little  
271 biological effects (Figure 2). The results also showed a response of the mussels that  
272 corresponded with the less contaminated station in Le Moulard (France) and the  
273 more contaminated site in Le Havre (France), both in the Seine estuary. At one site  
274 (Cartagena, SE Spain) there were elevated lead concentrations in the mussels, which  
275 did not appear to result in biological effects. In contrast, a high stress response  
276 (LMS) was observed at two sites (Firth of Forth in Scotland, Wadden Sea in the  
277 Netherlands) where concentrations of the measured contaminants were below EAC  
278 thresholds, suggesting alternative environmental stressors (not measured here) as  
279 the cause of the response. More focused monitoring would be required to determine  
280 the cause of the effects observed at those two sites.

281

282 The imposex response of gastropods to environmental concentrations of organotins  
283 has been integrated in the scheme by incorporating results from adjacent shoreline  
284 populations (Figure 3). Only a single site (Le Havre in the Seine estuary) had a level  
285 of imposex of concern, above EAC.

286

287 The fish species included in the assessment were dab (LL), flounder (PF), haddock  
288 (MA) and red mullet (MB). Two of the species were found at some sites, e.g. dab and  
289 haddock in the Firth of Forth and the two Iceland sites and dab and flounder in the  
290 Seine estuary and the Baltic site (Figure 4). Concentrations of PCBs in dab, flounder  
291 and haddock exceeded EACs at some sites and fish at all sites except red mullet at  
292 Cartagena had elevated concentrations of Cd. Furthermore, there was evidence of  
293 exposure of dab, flounder and haddock to PAHs at many sites, including  
294 Hvassahraun, Firth of Forth, German Bight, Wadden Sea, Seine sites and the Baltic  
295 site. There was good correspondence between results for the two methods used to  
296 quantify PAH metabolites, but no clear relationship between the elevated PAH  
297 metabolite concentrations at many locations and responses such as EROD and  
298 measures of genotoxicity (comet, DNA adducts). There were however values above  
299 EAC for both LMS and AChE at three sites, including Ekofisk, Dogger Bank and the  
300 Baltic site (all dab), and for one of them at Iceland (dab), Firth of Forth (dab), the  
301 Seine estuary (flounder) and the Baltic (flounder). Histology also suggested a range  
302 of sites were somewhat affected, i.e. dab at both Iceland sites, dab at Ekofisk,  
303 flounder at all Firth of Forth sites, dab at Firth of Forth, Dogger Bank and the  
304 German Bight.

305

### 306 **Assessment by site**

307 To allow region-wide assessments, data are combined by matrix and site. Such an  
308 assessment could include selected regions, e.g. Iceland, North Sea coastal and  
309 offshore, the Baltic and the Mediterranean. Figures are only shown for North Sea  
310 offshore to demonstrate what such an assessment may look like. Sites at Iceland  
311 included both coastal (Bjarnarhöfn, Hvassahraun) and offshore (Iceland SE, Iceland  
312 SW) locations. All determinants for the coastal sites were below EAC, whereas  
313 contaminants (PCB in haddock liver) and effects (AChE and DNA adducts in fish and

314 bioassays of whole sediments) were above EAC for one or more of the two offshore  
315 sites sampled. Most of the exposure responses were at or below background levels.  
316 Both contaminants and effects were above EAC at some coastal sites in the North  
317 Sea. Although coastal North Sea sites comprised the greatest data contribution to  
318 the overall assessment, there were biological responses lacking, particularly for  
319 exposure. Contaminant concentrations were largely below EAC levels in North Sea  
320 offshore sites, except for PCBs in fish liver at Firth of Forth and German Bight  
321 (Figure 5). At most sites there was evidence of exposure of fish to genotoxic  
322 compounds. At the sites Ekofisk, Firth of Forth and Dogger Bank there were  
323 significant levels (>EAC) of toxicant-induced physiological stress. At the single site  
324 surveyed in the Baltic there was evidence of contamination above background levels  
325 for PAH and heavy metals (Cd) with some heavy metals (Pb, Hg) exceeding EAC  
326 thresholds in sediment and PCBs exceeding EAC in dab livers. Dab was found to be  
327 exposed to PAH, and both flounder and dab showed significant effects through LMS  
328 (and AChE for flounder) effects indicators.

329

### 330 **Regional assessments**

331 Results of the assessments conducted above can be further aggregated into regional  
332 assessments by representing the proportion of determinant/matrix/site in each  
333 assessment category (blue, green, red). This can be visualised for contaminants,  
334 exposure and effects indicators as in Figure 6 or by combining the three in Figure 7.

335

336 For an area or region, Figure 7 shows that we have a simple aggregated assessment for  
337 all matrices, determinants and sites in a region with the relative proportion of all  
338 observations exceeding BAC and EAC. When considering suitable environmental targets  
339 for contaminants and their effects and the wording of Descriptor 8 in the Marine  
340 Strategy Framework Directive (MSFD), Good Environmental Status might be taken to  
341 mean that concentrations of contaminants and measurements of their effects should  
342 always be less than EAC. It should be borne in mind that when very large numbers of  
343 observations are made there is always the possibility that outliers are present and it  
344 would not be reasonable in such circumstances to have a 100% compliance target (or  
345 “one out all out”). Therefore SGIMC (ICES, 2011) proposed a pragmatic approach that

346 95% of measurements should be less than EAC (allowing for a 5% error rate). This  
347 target is represented as a horizontal red line in Figure 7.

348 **Discussion**

349 The assessment of the results from the ICON project shows that the framework  
350 provides a good and transparent reporting tool that makes it possible to present  
351 complex environmental monitoring datasets on contaminants concentrations and  
352 biological responses across multiple matrices, sites and seas. The key to the  
353 assessment is the development of the method- and species-specific criteria, which  
354 allows for the setting of thresholds of assumed equal significance for contaminants,  
355 exposure indicators and effect indicators, eventually allowing the different data  
356 types to be combined in a common indicator (cf. Vethaak et al., this issue-a). The  
357 flexibility and transparency is more extensive than frameworks proposed earlier,  
358 not least because contaminant concentrations and biological responses could be  
359 combined in a final assessment of environmental status. In addition, the ICON  
360 sampling campaign in European coastal and offshore areas provided a large dataset  
361 that resulted in a comprehensive and comparative evaluation of the state of selected  
362 European coastal and offshore marine areas.

363

364 The core methods included in the scheme were selected as the minimum set of  
365 contaminants and biological effects techniques that would need to be applied in  
366 order to determine whether contaminants are impacting on 'ecosystem health'.  
367 They achieve this by covering the main contaminant groups likely to cause such  
368 effects and that may be routinely monitored, as well as covering the main toxicity  
369 endpoints that are reasonably measurable in sentinel species, i.e. general toxicant  
370 stress, neurotoxicity, genotoxicity (Hylland et al., this issue-b), carcinogenicity (Lang  
371 et al., this issue-b), endocrine disruption (Burgeot et al., this issue), energetic costs  
372 (Martinez-Gomez et al., this issue-a) and mortality, as well as biomarkers of  
373 exposure to groups of compounds likely to have such effects. This core set of  
374 methods is not identical to, but similar to those suggested by under HELCOM  
375 (Lehtonen et al., 2014), but more extensive than methods suggested in e.g. Giltrap et  
376 al. (2013) and Hagger et al. (2008). Sediment bioassays are not mandatory in the  
377 OSPAR framework, but should comprise more than one method (as reported here).  
378 Sediment toxicity was addressed using different methods in Vethaak et al. (this issue  
379 – b).

380

381 There are environmental factors that may modulate biological responses, e.g.  
382 season. Data used to derive BAC and EAC were from studies where ICES guidelines  
383 for sampling have been adhered to, i.e. sampling outside the reproductive period.  
384 Criteria have been developed for selected species using hundreds and thousands of  
385 analyses as a basis, but there is an underlying assumption in this strategy that a  
386 species will respond to contaminant exposure in a similar fashion throughout its  
387 geographical range, all else being equal.

388

389 The biological responses selected for the framework comprise a range of methods  
390 that are sensitive to contaminant stress, including some that are specific to  
391 important contaminant groups and some that provide responses to a wide range of  
392 substances, including cumulative effects and effects from chemicals not directly  
393 monitored for. The integrated nature of the approach also identified instances  
394 where high concentrations of contaminants of concern were recorded, but where  
395 effects were not detected at a significant level. In these instances, contaminant  
396 availability may be limited and concentrations of limited concern as a result. In this  
397 case, the lack of effects in the assessment will down-weight the importance of the  
398 contaminant result in an overall assessment. If the 95% target were to be used as a  
399 regional indicator of MSFD GES, Iceland and offshore North Sea would achieve the  
400 target using the ICON dataset, but inshore North Sea, Baltic and Spanish  
401 Mediterranean regions would fail.

402

403 Through applying the integrated assessment framework to the ICON dataset, several  
404 issues were identified that will need to be considered or spawn further research to  
405 improve the robustness of the framework. Because the assessment approach largely  
406 aggregates the results of applying thresholds to monitoring data at various levels of  
407 organisation and spatial scales, all data are treated equally in the assessment  
408 process and missing data will necessarily introduce less robustness into the overall  
409 assessment. Similarly, the introduction of additional data, for example from multiple  
410 matrices of the same type, e.g. multiple species of fish at the same site, can skew the  
411 assessment result. The ICON project has demonstrated that even on the scale of a  
412 large project with more than 20 partner institutions, data are likely to be missing  
413 from an assessment. In the current report, this has been dealt with by the use of



414 'grey' in the figures, so that the uncertainty of an assessment can be identified. It is  
415 further recommended that a 'robustness indicator' be developed in order to be able  
416 to quantify the quality of site assessments (see Martinez-Gomez et al., this volume –  
417 b). Such an indicator would be based on the relevance and completeness of the  
418 range of determinants comprising an assessment. Finally, the outcome of any  
419 integrated assessment has the potential to be strongly influenced by the selection of  
420 sites for the programme. At present there are no guidelines recommending a  
421 minimum number of sampling sites per region, appropriate statistical power for  
422 monitoring using this approach or how to account for hotspot or inshore sites in a  
423 wider scale regional assessment. Those are issues that need to be addressed to  
424 ascertain relevant and efficient marine monitoring in the future.

425

## 426 **Conclusions**

427 The ICON project has provided one of the most comprehensive integrated  
428 monitoring datasets of its kind and was found to be suitable for assessment using  
429 the framework developed within ICES and OSPAR. The approach is considered  
430 suitable for the determination of GES for Descriptor 8 under the MSFD.

431

432 The ICON project has shown that it is feasible to apply the OSPAR framework for  
433 integrated chemical and biological monitoring. The results show that Iceland has  
434 locations less impacted by contaminants than other locations in Europe, followed by  
435 offshore locations in the North Sea, with coastal locations being most clearly  
436 impacted.

437

438 The framework can be applied to datasets with missing data and determinants, but  
439 the validity of the assessment decreases with increasing missing data. Further  
440 guidance on minimal requirements for an integrated assessment and the  
441 development of a robustness indicator is suggested.

442

443 Assessment criteria for passive sampling techniques and *in vitro* bioassays need  
444 further development before they can be included in the integrated assessment  
445 framework.

446

447 There is a need to evaluate some assumptions in the OSPAR framework, e.g. that  
448 different populations of a species with a wide geographical coverage will respond  
449 similarly to contaminant exposure.

450

451

452 **Acknowledgements**

453 The authors wish to acknowledge the work by colleagues in ICES and OSPAR working  
454 group, i.e. WGBEC, WKIMON, SGIMC, as well as the cruise leaders, cruise participants  
455 and crews of R/V Walther Herwig III (Germany), R/V Scotia, R/V Alba na Mara  
456 (Scotland), R/V Gwen Drez (France) and R/V Endeavour (UK). The French participation  
457 was funded by IFREMER and ONEMA.

458

459

460 **Literature references**

461

462 Adams, S.M., Brown, A.M., Goede, R.W., 1993. A quantitative Health Assessment Index for rapid  
463 evaluation of fish condition in the field. Transactions of the American Fisheries Society, 122, 63-  
464 73.

465 Allen, Y., Scott, A. P., Matthiessen, P., Haworth, S., Thain, J. E., Feist, S. 1999. Survey of estrogenic  
466 activity in United Kingdom estuarine and coastal waters and its effects on gonadal development  
467 of the flounder *Platichthys flesus*. Environmental Toxicology and Chemistry, 18, 1791–1800.

468 Bocquene, G., Galgani, F., Burgeot, T., Le-Dean, L., Truquet, P., 1993. Acetylcholinesterase levels  
469 in marine organisms along French coasts. Marine Pollution Bulletin, 26, 101–106.

470 Broeg, K., von Westernhagen, H., Zander, S., Körting, W., Koehler, A., 2005. The biological  
471 assessment index (BAI) a concept for the quantification of effects of marine pollution by an  
472 integrated biomarker approach. Marine Pollution Bulletin, 50, 495-503.

473 Bourlat, S.J., Borja, A., Gilbert, J., Taylor, M.I., Davies, N., Weisberg, S.B., Griffith, J.F., Lettieri, T.,  
474 Field, D., Benzie, J., Glöckner, F.O., Rodríguez-Ezpeleta, N., Faith, D.P., Bean, T.P., Obst, M., 2013.  
475 Genomics in marine monitoring: New opportunities for assessing marine health status. Marine  
476 Pollution Bulletin, 74, 19–31.

477 Carney Almroth, B., Hultman, M., Wassmur, B., Sturve, J., Is oxidative stress evident in dab  
478 (*Limanda limanda*) in the North Sea? (this issue)

479 Chapman, P.M., McDonald, B.G., Lawrence, G.S., 2002. Weight-of-evidence issues and frameworks  
480 for sediment quality (and other) assessments. Human and Ecological Risk Assessment 8, 1489–  
481 1515.

482 Dagnino, A., Allen, J.I., Moore, M.N., Broeg, K., Canesi, L., Viarengo, A., 2007. Development of an  
483 expert system for the integration of biomarker responses in mussels into an animal health index.  
484 Biomarkers, 12, 155-172.

485 Davies, I.M., Vethaak, A.D. (Eds.), 2012. Integrated monitoring of chemicals and their effects. ICES  
486 Cooperative Research Report 315, 227 pp.

487 Devin, S., Burgeot, T., Giamberini, L., Minguez, L., Pain-Devin, S., 2014. The integrated biomarker  
488 response revisited: optimization to avoid misuse. Environmental Science And Pollution  
489 Research, 21, 2448-2454.

490 Giltrap, M., Ronan, J., Hardenberg, S., Parkes, G., McHugh, B., McGovern, E., Wilson, J.G., 2013.  
491 Assessment of biomarkers in *Mytilus edulis* to determine good environmental status for  
492 implementation of MSFD in Ireland. *Marine Pollution Bulletin* 71, 240–249.

493 Hagger, J.A., Jones, M.B., Lowe, D., Leonard, D.R.P., Owen, R., Galloway, T.S., 2008. Application of  
494 biomarkers for improving risk assessments of chemicals under the Water Framework Directive:  
495 A case study. *Marine Pollution Bulletin*, 56, 1111–1118.

496 Hylland, K., Beyer, J., Berntssen, M., Klungsøyr, J., Lang, T., Balk, L. 2006. May persistent organic  
497 pollutants affect fish populations in the North Sea? *Journal of Toxicology and Environmental*  
498 *Health, Part A*, 69, 125-138.

499 Hylland, K., Gubbins, M.J., Robinson, C., Burgeot, T., Martínez-Gómez, C., Lang, T., Svavarsson, J.,  
500 Thain, J.E., Vethaak AD. Integrated chemical and biological assessment of contaminant impacts in  
501 selected European coastal and offshore marine areas (this issue-a)

502 Hylland, K., Ruus, A., Grung, M., Green, N., 2009. Relationships between physiology, tissue  
503 contaminants and biomarker responses in Atlantic cod (*Gadus morhua* L.). *Journal of Toxicology*  
504 *and Environmental Health, Part A*, 72, 226-233.

505 Hylland, K., Skei, B.B., Gubbins, M.J., Lang, T., Brunborg, G., Le Goff, J., Burgeot, T., Genotoxicity in  
506 dab (*Limanda limanda*) and haddock (*Melanogrammus aeglefinus*) from European seas (this  
507 issue-b)

508 Hylland, K., Tollefsen, K.-E., Ruus, A., Jonsson, G., Sundt, R.C., Sanni, S., Utvik, T.I.R., Johnsen, S.,  
509 Nilssen, I., Pinturier, L., 2008. Water column monitoring near oil installations in the North Sea  
510 2001–2004. *Marine Pollution Bulletin*, 56, 414–429.

511 ICES, 2011. Report of the Study Group on Integrated Monitoring of Contaminants and Biological  
512 Effects (SGIMC), 14–18 March 2011, Copenhagen, Denmark. ICES CM 2011/ACOM:30. 265 pp.

513 Kammann, U., Akcha, F., Budzinski, H., Burgeot, T., Gubbins, M.J., Lang, T., Le Menach, K., Vethaak,  
514 A.D., Hylland, K. PAH metabolites in fish bile: from the Seine Estuary to Iceland (this issue)

515 Lang, T., Feist, S.W., Stentiford, G.D., Bignell, J., Vethaak, A.D., Wosniok, W. Diseases of dab  
516 (*Limanda limanda*): analysis and assessment of data on externally visible diseases, macroscopic  
517 liver neoplasms and liver histopathology at offshore sites in the North Sea, Baltic Sea and off  
518 Iceland (this issue-a)

519 Lang, T., Kruse, R., Haarich, M., Wosniok, W. Methylmercury in dab (*Limanda limanda*) from the  
520 North Sea, Baltic Sea and Icelandic waters: relationship to host-specific variables (this issue-b)

521 Lyons, B., Thain, J.E., Stentiford, G.D., Hylland, K., Davies, I., Vethaak, A.D. 2010. Using biological  
522 effects tools to define Good Environmental Status under the European Union Marine Strategy  
523 Framework Directive. *Marine Pollution Bulletin*, 60, 1647-1651.

524 Lyons B.P., Bignell, J.P., Stentiford, G.D., Bolam, T., Rumney, H.S., Bersuder, P., Barber, J., Askem,  
525 C.W., Maes T., Thain, J.E. Determining Good Environmental Status under the Marine Strategy  
526 Framework Directive: case study for descriptor 8 (chemical contaminants) (this issue)

527 Marigómez, I., Garmendia, L., Soto, M., Orbea, A., Izagirre, U., Cajaraville, M.P., 2013. Marine  
528 ecosystem health status assessment through integrative biomarker indices: a comparative study  
529 after the Prestige oil spill “Mussel Watch”. *Ecotoxicology*, 22, 486–505.

530 Martínez-Gómez C., Burgeot T., Robinson, C.D., Gubbins, M.J., Halldorsson, H.P. Albentosa, M.,  
531 Bignell J.P., Hylland, K., Vethaak A.D. Lysosomal membrane stability and Stress on Stress in  
532 mussels as common Pan-European contaminant-related biomarkers (this issue-a)

533 Martínez-Gómez C., Fernández B., Robinson, C.D., Campillo J.A., León V.M., Benedicto J., Hylland,  
534 K., Vethaak A.D. Assessing the good environmental status (GES) of the Cartagena coastal zone (W  
535 Mediterranean) using an integrated framework of chemical and biological effect data: a practical  
536 case study (this issue-b)

537 OSPAR, 2008. Draft Agreement on CEMP Assessment Criteria for the QSR 2010, , MON 09/8/1/6  
538 Add.1. OSPAR Commission, London.

539 OSPAR, 2010. Quality Status Report 2010. OSPAR Commission, London, 176 pp.

540 Robinson, C.D., Webster, L., Martínez-Gómez, C., Burgeot, T., Gubbins, M.J., Thain, J.E., Vethaak,  
541 A.D., McIntosh, A.D., Hylland, K. Assessment of contaminant concentrations in sediments, fish  
542 and mussels sampled from the North Atlantic and European regional seas within the ICON  
543 project (this issue)

544 Sandstrom, O., Larsson, A., Andersson, J., Appelberg, M., Bignert, A., Ek, H., Forlin, L., Olsson, M.,  
545 2005. Three decades of Swedish experience demonstrates the need for integrated long-term  
546 monitoring of fish in marine coastal areas. *Water Quality Research Journal of Canada*, 40, 233–  
547 250.

548 Thain, J.E., Vethaak, A.D., Hylland, K., 2008. Contaminants in marine ecosystems: developing an  
549 integrated indicator framework using biological effects techniques. *ICES Journal of Marine  
550 Science*, 65, 1508-1514.

551 Tornero, V., d’ Alcalà, M.R., 2014. Contamination by hazardous substances in the Gulf of Naples  
552 and nearby coastal areas: A review of sources, environmental levels and potential impacts in the  
553 MSFD perspective. *Science of the Total Environment* 466, 820–840.

554 Vethaak, A.D., Davies, I.M., Thain, J.E., Gubbins, M.J., Martínez-Gómez, C., Robinson, C.D., Moffat,  
555 C.F., Burgeot, Maes, Wosniok, Giltrap, M., Lang, T., Strand, J., Hylland, K. Integrated indicator  
556 framework and methodology for monitoring and assessment of hazardous substances and their  
557 effects in the marine environment (this issue-a)

558 Vethaak, A.D., Hamers, T., Martínez-Gómez, C., Kamstra, J.H., de Weert, J., Leonards, P., Smedes, F.  
559 Toxicity profiling of marine surface sediments: a case study using rapid screening bioassays of  
560 exhaustive total extracts, elutriates and passive sampler extracts (this issue-b)

561 Vethaak, A. D., Jol, J. G., Martínez-Gómez, C., 2011. Effects of cumulative stress on fish health near  
562 freshwater outlet sluices into the sea: a case study (1988–2005) with evidence for a contributing  
563 role of chemical contaminants. *Integrated environmental assessment and management*, 7, 445-  
564 458.

565

566 **Figure captions**

567

568 Figure 1. Assessment of sediment data against BAC (background assessment criteria)  
569 and EAC (ecotoxicological assessment criteria); blue - below BAC, green - between BAC  
570 and EAC, red - above EAC, grey – data lacking; FoF = Firth of Forth.

571

572 Figure 2. Assessment of mussel data against BAC (background assessment criteria)  
573 and EAC (ecotoxicological assessment criteria); blue - below BAC, green - between  
574 BAC and EAC, red - above EAC; grey cells indicate core analyses not performed.

575

576 Figure 3. Assessment of imposex data (as VDSI) against BAC (background assessment  
577 criteria) and EAC (ecotoxicological assessment criteria); blue - below BAC, green -  
578 between BAC and EAC, red - above EAC; grey cells indicate analyses not performed.

579

580 Figure 4. Assessment of contaminant concentrations (liver), exposure and effects in fish  
581 from Iceland, the North Sea, Baltic Sea, Seine estuary (two sites) and Mediterranean Sea;  
582 LL – dab, PF – flounder, MA – haddock, MB - red mullet; blue - below BAC, green -  
583 between BAC and EAC, red - above EAC; grey cells indicate core analyses not performed;  
584 see Davies & Vethaak (2012) and relevant chapters for individual methods.

585

586 Figure 5. Assessment of contaminants, exposure and effects for the indicated locations in  
587 the North Sea (offshore); grey cells indicate core analyses not performed.

588

589 Figure 6. Assessment of contaminants, exposure and effects for each of the five areas.  
590 From left: Iceland (4 sites), coastal North Sea (10 sites), offshore North Sea (5 sites),  
591 German Baltic Sea (1 site) and Spanish Mediterranean Sea (2 sites). Numbers indicate  
592 data for each category.

593

594 Figure 7. Integrated assessment for each of the five areas. From left: Iceland (4 sites),  
595 coastal North Sea (10 sites), offshore North Sea (5 sites), German Baltic Sea (1 site) and  
596 Spanish Mediterranean Sea (2 sites). Numbers indicate data for each category; red line =  
597 95% threshold.

598