Assessment of contaminant concentrations in sediments, fish and mussels sampled from the North Atlantic and European regional seas within the ICON project

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Abstract :

Understanding the status of contaminants in the marine environment is a requirement of European Union Directives and the Regional Seas Conventions, so that measures to reduce pollution can be identified and their efficacy assessed. The international ICON workshop (Hylland et al., in this issue) was developed in order to test an integrated approach to assessing both contaminant concentrations and their effects. This paper describes and assesses the concentrations of trace metals, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls in sediments, mussels, and fish collected from estuarine, coastal and offshore waters from Iceland to the Mediterranean Sea. For organic contaminants, concentrations progressively increased from Iceland, to the offshore North Sea, to the coastal seas, and were highest in estuaries. Metals had a more complex distribution, reflecting local anthropogenic inputs, natural sources and hydrological conditions. Use of internationally recognised assessment criteria indicated that at no site were concentrations of all contaminants at background and that concentrations of some contaminants were of significant concern in all areas, except the central North Sea.

Graphical abstract
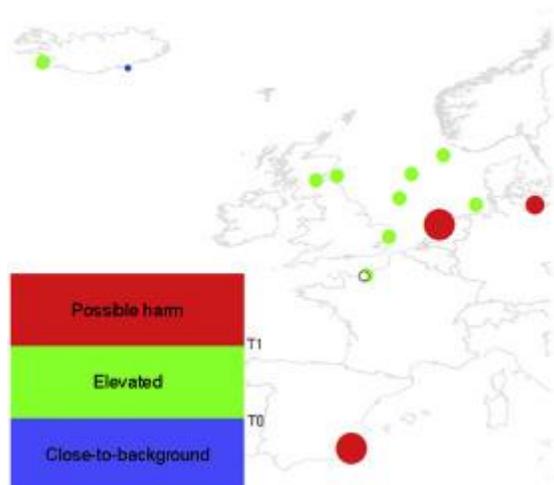
Highlights

► Sediments, fish and mussels were collected from Iceland to the Mediterranean Sea. ► Concentrations of organics were lowest at Iceland and highest in the Seine estuary. ► Concentrations of metals were highest at Cartagena in the Mediterranean Sea. ► At most sites, concentrations of at least one contaminant were of significant concern.

Keywords : Integrated assessment, ICES, North Atlantic, North Sea, Baltic Sea, Mediterranean Sea, Polycyclic aromatic hydrocarbons (PAHs), Polychlorinated biphenyls (PCBs), Trace metals, Environmental monitoring

1. Introduction

The monitoring and assessment of contaminants in European seas has been undertaken for many years to satisfy the requirements of the Regional Seas Conventions (RSCs), i.e. OSPAR for the NE Atlantic (including the North Sea), HELCOM for the Baltic Sea, MED POL for the Mediterranean Sea. From 2014, Descriptor 8 of the European Union Marine Strategy Framework Directive (MSFD; EU, 2008a) requires the monitoring of contaminants within regular assessment cycles and aims to achieve Good Environmental Status for European seas by 2020. States with coasts in more than one Regional Sea (e.g. France, Spain), have an

76 operational requirement to harmonise approaches between RSCs and are making
77 use of advice from the International Council for the Exploration of the Sea (ICES) to
78 do this.
79
80 OSPAR Contracting Parties are committed to participate in its Coordinated
81 Environmental Monitoring Programme (CEMP), under which a number of
82 contaminants and biological effects are either mandatory, or voluntary determinants.
83 Mandatory CEMP determinants include polycyclic aromatic hydrocarbons (PAHs) in
84 sediment and shellfish, polychlorinated biphenyls (PCBs), polybrominated biphenyl
85 ethers (PBDEs) and hexabromocyclododecane (HBCDD) in sediments, fish liver and
86 shellfish, the toxic metals Cd, Hg and Pb in sediments and biota, and endocrine
87 disruption (imposex) in gastropod molluscs.   Voluntary (pre-CEMP) chemical
88 determinants include chlorinated dioxins and furans, alkylated-PAHs, polyfluorinated
89 compounds (PFC)s in sediment and biota, and PFCs in water. Pre-CEMP biological
90 effects determinants include metals-specific effects, PAH-specific effects, and
91 general biological effects (OSPAR, 2010a). Through its regional coordination role
92 under the MSFD, OSPAR has made recommendations regarding the use of common
93 and candidate indicators for monitoring under Descriptor 8 (Concentrations of
94 contaminants are at levels not giving rise to pollution effects). Common indicators
95 include metals, organotins, PAHs, PCBs, and PBDEs in sediments, metals and
96 PCBs in fish, and metals, PAHs and PCBs in mussels; candidate indicators include
97 biological effects, i.e. lysosomal membrane stability, external fish disease,
98 cytochrome P450 (EROD) activity and biliary PAH metabolite concentrations
99 (OSPAR, 2013).   The current advice from the International Council for the
100 Exploration of the Seas (ICES) is that the monitoring and assessment of
101 environmental contaminants are fully integrated, with both contaminant
102 concentrations and their biological effects being monitored, reported, and assessed
103 in a coherent manner (Davies and Vethaak, 2012; Vethaak *et al.,* this issue). In the
104 North Atlantic, OSPAR has developed criteria (background assessment
105 concentrations (BACs) and environmental assessment concentrations, EACs) to
106 assess the significance of observed contaminant concentrations (OSPAR, 2009),
107 although under the MSFD European Environmental Quality Standards (EQSs)
108 should be used where they are available (e.g. for some contaminants in biota).
109 Similarly, regionally-specific assessment criteria are available for use in the
110 Mediterranean (UNEP/MAP, 2011; Angelidis *et al.,* 2011; Benedicto *et al.,* 2012; see
111 also Martínez-Gómez *et al.,* this issue). During the ICES advice drafting stage, a
112 multipartner project to examine the integrated approach to environmental
113 assessment at the regional seas level was conducted (International Workshop on
114 Integrated Assessment of Contaminant Impacts on the North Sea, ICON; Hylland *et*
115 *al.,* this issue). Our paper describes and assesses the contaminant concentrations
116 of sediment and biota samples collected from Iceland, the North Sea, the Baltic Sea,
117 and the Mediterranean Sea during the ICON project. The exposure of ICON fish
118 samples to PAHs is assessed in an accompanying paper on bile metabolites
119 (Kammann *et al.,* this issue).
120
121
122 2.   Material and methods
123 2.1.   Sample collection
124 Samples were collected by shoreline sampling (mussels; 2008-2011), or from
125 research cruises (fish, sediments) conducted in 2008 and 2009 by the participating

126 laboratories utilising the research vessels *Scotia, Alba na Mara* (both Marine
127 Scotland, UK), *Walter Herwig III* (Thünen-Institut für Fischereiökologie*,* Germany),
128 *Francisco de Paula Navarro* (Instituto Español de Oceanografía (IEO), Spain) and
129 *Gwendrez* (Ifremer, France). Fish and sediments from the Wadden Sea were
130 collected from a small boat, with surface sediments collected by van Veen grab and
131 flounder collected using a 3 m beam trawl. Mussels were hand collected between
132 September and February and not during the spawning period in order that the
133 animals had normal condition and lipid level. All sampling was in accordance with
134 OSPAR guidelines (OSPAR, 1999). After collection, samples for chemical analysis
135 were stored frozen (-20° C) and dispatched on dry ice to Marine Scotland Science
136 (MSS), Aberdeen, UK. Sampling site details are in Table 1 and locations are shown
137 in Figure 1. Occasional samples were lost or were found unsuitable for analysis; in
138 such cases alternative data (from the same year) were obtained from national
139 monitoring programs when they were available. To present and assess national
140 data consistently with ICON data, a conversion factor of 5 was used to convert
141 between mussel dry weight and wet weight where the moisture content was not
142 available.

143

144 The upper 2 cm of sediment were collected (e.g. by Day or van Veen grab),
145 thoroughly mixed and then frozen at -20° C as separate aliquots for the
146 determination of organic and inorganic compounds. Fish were collected by bottom
147 or beam trawls, examined for external disease and dissected at sea for tissue
148 sampling. Otoliths were removed and each fish subsequently aged by
149 sclerochronology, except red mullet which were in the size range 12-18 cm and age
150 was estimated as being Class I-III using specific size-age models (Kinacigil *et al.*,
151 2001; Martínez-Gómez *et al.,* this issue). In order to provide sufficient material for
152 the chemical analyses, liver samples were pooled (5 pools of 5 same-sex fish per
153 site for metals), minced and thoroughly mixed before being divided into aliquots for
154 organic or metals analyses and frozen at -20° C; flesh samples were treated
155 similarly, though for metals analysis only. After being defrosted and drained of
156 liquor, mussel soft tissues were removed from the shells, pooled, homogenised
157 using an ultra-Turrex homogeniser (VWR, Leicestershire, UK) and separate aliquots
158 frozen for subsequent determinations of trace metals or organic contaminants. For
159 PCB analyses, the 5 fish liver pools from each site were further combined according
160 to their sex and homogenised such that two determinations of liver PCB
161 concentrations were undertaken *per* site and species. Analysis of contaminants in
162 red mullet was conducted in muscle fish tissue, following guidelines of the MED POL
163 programme (UNEP/FAO/IOC/IAEA, 1993).

164

165 At most sites the collected mussels were from natural populations, although those
166 from the sampling sites at Cape Palos and Cartagena (SE Spain), had been
167 transplanted there (for 6 weeks) from a reference site located in open waters near
168 Malaga, SE Spain (see Martínez-Gómez *et al.,* this issue). From each mussel
169 sampling site, at least 20 individuals of a given size range (usually 45±10 mm) were
170 hand collected from below the water surface, close to the low water mark, and
171 transported cool prior to frozen storage.

172

173 At all stages, appropriate care was taken to avoid adventitious contamination of the
174 sample and losses of analytes, including the use of acid-washed non-metallic tools
175 and containers when handling samples for metals analyses and the use of solvent-

176 washed metallic or glass materials when handling samples for the determination of
177 organic contaminants.
178
179
180 2.2.   Trace metals analyses
181 2.2.1. Sample digestion
182 Sediment samples were freeze-dried, passed through a 2 mm sieve, ground with a
183 pestle and mortar, and digested using a Multiwave 3000 Microwave Digestion
184 System (Anton Paar, St Albans, UK). Approximately 250 mg (± 50 mg) of powdered
185 sediment was digested using 2 mL of hydrogen peroxide, 1.5 mL of hydrofluoric and
186 5 mL of nitric acid (VWR Merck, Lutterworth, UK); the digestion programme was 15
187 mins ramp to 200° C, hold for 25 mins and cool for 30 mins; excess HF was
188 complexed with 10 mL of saturated boric acid and microwave heating.
189 Homogenised biota samples were also digested using the Multiwave 3000; either
190 600 ± 100 mg (wet fish flesh), 450 ± 50 mg (wet liver, wet mussels), or 250 ± 50 mg
191 (dried Certified Reference Material, CRM) were digested using 2.5 mL nitric acid and
192 3.5 mL hydrogen peroxide, and a digestion programme of ramp to 70° C in 2
193 minutes, hold for 5 mins, ramp to 200°C in 13 mins , hold for 25 minutes and cool for
194 30 mins. Each digestion run included one Certified Reference Material (CRM) and
195 one procedural blank; after digestion, each sample was diluted to 25 mL using ultra-
196 pure water (>18 MΩ.cm; Elga, Marlow, UK). The following CRMs (NRCC, Canada)
197 were analysed: MESS-3 marine sediment, TORT-2 shellfish hepatopancreas,
198 DORM-3 fish muscle and DOLT-3 fish liver.
199
200 2.2.2. Metals analysis
201 The digests were further diluted (20-fold for sediments, 5-fold for biota) with 1% nitric
202 acid containing internal standards (Rh, Ir, Sc, Ge, Bi), Au and 1% thiourea. Au and
203 thiourea (biota only) were used to reduce memory effects in the determination of Hg.
204 Concentrations of trace elements (As, Cu, Zn, Cd, Hg, Pb) were determined using
205 matrix-matched calibration standards and an Elan 6100*DRC*+ inductively coupled
206 plasma mass spectrometer (ICPMS; Perkin Elmer, Buckinghamshire, UK) equipped
207 with a cross-flow nebuliser and Scott spray chamber.   Additional elements
208 determined included Fe and Al (sediments) and Se (biota). Tuning solutions and
209 analytical standards were obtained from Spex CertiPrep, Stanmore, Middlesex, UK.
210
211 2.3.   Organic contaminants
212 2.3.1. Polychlorinated biphenyls
213 PCBs were determined according to Webster *et al.* (2011). Briefly, 10-20 g of dried
214 sediment or 0.5-2 g of wet tissue were extracted with *iso*-hexane using pressurised
215 liquid extraction (Dionex Ltd., Camberley, Surrey, UK,) and deactivated silica clean-
216 up. Concentrations of 32 PCB congeners (including the "ICES-7" indicator PCBs,
217 CBs 28, 52, 101, 118, 138, 153 and 180) were determined by gas chromatography
218 with electron capture detection (GC-ECD).   When summing congener
219 concentrations, zero was used in place of values below the detection limit (LoD), and
220 a value of half the limit of quantification (LoQ) was used when trace concentrations
221 were too low to be quantified.
222
223 2.3.2. Polycyclic aromatic hydrocarbons
224 PAHs were determined in sediment and mussels according to Webster *et al.* (2009
225 and 2011). Briefly, deuterated internal standards were added to approximately 20 g

226 of wet sediment which was then sonicated in 40 mL dichloromethane/methanol (1:1
227 v/v) for 5 mins and centrifuged to remove the solids. The supernatant was liquid-
228 liquid extracted and dried with sodium sulphate. The extraction was repeated, the
229 extracts were combined, solvent exchanged into *iso*-hexane and concentrated by
230 rotary evaporation. The aliphatic and aromatic fractions were separated by isocratic
231 high performance liquid chromatography (HPLC) and the aromatic fraction further
232 concentrated (to approx. 300 µL) before the concentrations of 40 parent and
233 alkylated PAHs were determined by gas chromatography and mass spectrometry
234 (GCMS) in selective ion monitoring mode. Mussels (approx. 10 g wet wt) were
235 extracted by saponification with methanol and sodium hydroxide in water for 4 hours
236 and extracted with *iso*-hexane/methanol/water (4:1:1). The extracts were
237 concentrated, purified and analysed as for sediments.
238
239 2.4.   Supporting determinants
240 The proportions of fine grained material (%<63 µm and %<20 µm) in freeze-dried
241 sediments initially sieved to <2 mm were determined by laser granulometry
242 (Mastersizer 2000; Malvern Instruments, Worcestershire, UK) and the total organic
243 carbon (TOC) content was determined using a ThermoQuest FlashEA 1112
244 elemental analyser (Thermo Scientific, Hemel Hempstead, UK) following acidification
245 of the sediment with 15% hydrochloric acid to remove inorganic carbonate.
246
247 2.5.   Quality Control
248 Analyses at MSS were conducted within a laboratory accredited to ISO-17025 by the
249 UK Accreditation Service (UKAS) for the determinations of metals, PCBs, PAHs,
250 particle size and TOC in marine sediment and/or biota. All analytical batches
251 included the analysis of Certified or Laboratory Reference Materials (LRMs), with the
252 results recorded on Shewart control charts with warning and control limits set at two-
253 and three-times standard deviation respectively. External quality assurance was
254 confirmed through successful participation in the QUASIMEME proficiency testing
255 scheme.
256
257 2.6.   Data analysis and assessment
258 All statistical analyses were performed using Minitab v16. Patterns of contaminant
259 concentration and composition were investigated using principle components
260 analysis (PCA). Where sufficient data were available (e.g. metals in fish liver), one-
261 way ANOVA and Tukey's *post hoc* tests were conducted on data (log-transformed
262 where required) from fish of the same species in order to investigate between-site
263 differences in concentration; non-parametric data were analysed by the Kruskall-
264 Wallis ANOVA by ranks and Dunn's *post hoc* test (Orlich, 2000) using a macro
265 available from http://support.minitab.com.
266
267 To assess the significance of determined contaminant concentrations, data were
268 compared with internationally recognised assessment criteria (Table 2) and
269 presented using a "traffic light" system, where blue indicated concentrations at
270 background, green indicated elevated concentrations and red indicated unacceptably
271 high concentrations (Fig. 2). The assessment followed the OSPAR approach (2008)
272 using Background Assessment Concentrations (BACs) for the blue/green transition
273 (T_0) and Environmental Assessment Criteria (EACs) for the green/red (T_1) transition,
274 with missing EACs being replaced by using other established criteria such as US
275 Sediment Quality Guidelines (Effects Range Low, ERLs; NOAA, 1999) or the

276 Maximum Permitted Concentrations (MPCs) under EU food regulations
277 (EC/1881/2006, as amended). OSPAR BACs/EACs for sediment are normalised to
278 5% Al (metals) or 2.5% organic carbon (organic contaminants), whilst ERLs are for
279 bulk sediment concentrations; we compared normalised or non-normalised sediment
280 concentrations as appropriate for the definition of the respective assessment criteria.
281 For the Mediterranean, MED POL has developed BACs for some contaminants (e.g.
282 metals in sediments; UNEP/MAP, 2011) whilst Spain has developed others for use in
283 biota for its Mediterranean waters (Benedicto *et al.,* 2011). MED POL has not yet
284 developed EACs for its region, but suggests using the OSPAR approach (i.e. using
285 OSPAR EACs, ERLs, or MPCs; UNEP/MAP, 2011). To date, neither OSPAR nor
286 MED POL intend to use EU Environmental Quality Standards (EQSs) for the
287 green/red transition (T_1) in biota (e.g. for Hg, fluoranthene, benzo[*a*]pyrene). In this
288 paper we have used the biota EQSs for Hg, fluoranthene and benzo[*a*]pyrene as
289 they are legal standards intended to protect the ecosystems of saline waters.
290
291 Mean concentrations and their 95% confidence intervals were compared with the
292 Assessment Criteria (OSPAR, 2008); where statistical confidence intervals were not
293 available, the analytical result and its uncertainty were compared to the Assessment
294 Criteria. In order to avoid being overly protective, individual organic contaminants
295 were assessed against their respective assessment criteria and two or more PAH
296 compounds or PCB congeners had to exceed a transition threshold before the site
297 was assessed as having exceeded that threshold, so for a site to be "red" for PAHs
298 in sediment, concentrations of at least two PAH compounds had to exceed their
299 individual ERL values.
300
301 3. Results and Discussion
302
303 3.1. Trace metals
304 A plot of the first two Principle Components from a PCA of sediment metal
305 concentrations (Fig. 3) grouped the five offshore sites (Egersund Bank, Ekofisk,
306 offshore Firth of Forth, Dogger Bank and German Bight) and the Alde estuary closely
307 together (with low metal concentrations), whilst the Wadden Sea and, especially,
308 Cartagena separated due to higher metal concentrations; the former is likely due to
309 inputs from the River Rhine (Bakker *et al.,* 2009), whilst mining wastes and Hg
310 biogeochemical cycling probably account for the higher metals concentrations in SE
311 Spain (Benedicto *et al.,* 2008, 2011; Cossa and Coquery, 2005). The Blackness,
312 Baltic Sea and Seine estuary sites formed a separate group with moderate
313 responses on both the first (PC1) and second (PC2) principle components, whilst the
314 SE Iceland (IS1) site was further separated on PC2 (relatively high for Cu and low for
315 As and Pb). As well as in sediments (Table S1; Figs. S1-S3), concentrations of Hg,
316 Zn, Cd and Pb were high in biota from Cartagena compared to the other sites
317 (Tables S4 and S5; Fig.s S4-S6), which is consistent with previous reports that the
318 area is one of the most heavily metal polluted areas of the western Mediterranean
319 Sea, due to marine dispersal of mining waste from the nearby Portmán bay area
320 (Benedicto *et al.,* 2008, 2011). Markedly high concentrations of Zn, Pb, Hg and Cd
321 have been reported in sediment and/or biota (Benedicto *et al.,* 2005, 2008, 2011), as
322 well as an acute toxicity to invertebrates exposed to sediments from this area (Cesar
323 *et al.,* 2004). Sublethal contaminant-related effects have been described in mussels
324 and red mullet from the surroundings of Cartagena marine area (Martínez-Gómez *et*
325 *al.,* 2008, 2012, this issue; Fernández *et al.,* 2010, 2011).

326
327 Sediment cadmium concentrations (Tables S1-S3) did not exceed the ERL at any
328 site, and were close-to-background in the Alde estuary (SE England) and in the
329 central North Sea (Fig. S1.). In contrast to sediments, Cd concentrations tended to
330 be higher in livers of dab from deeper, offshore, sites than from inshore areas (Table
331 S5; Fig. S4 & S5), but were also high at SE Iceland (IS1; 905 µg /kg) and SW
332 Iceland (IS2; 514 µg /kg). The differences in Cd concentrations were not related to
333 age as fish from Ekofisk, offshore Firth of Forth, Dogger Bank, German Bight and the
334 Baltic Sea sites were all approximately 2.5 years old, but had contrasting liver Cd
335 concentrations (approx. 1300 µg/kg w.w at Ekofisk, approx. 600 µg/kg w.w. at the
336 offshore locations Firth of Forth and Dogger Bank, in contrast to ca.150 µg/kg w.w at
337 the German Bight and the Baltic Sea sites). Flounder were only collected from
338 inshore and estuarine waters. In liver, Cd concentrations were highest at the
339 Parfond site in the Seine (ANOVA, *p*<0.001). High Cd concentrations in mussels
340 from the Seine estuary have also been reported previously (Chiffoleau *et al.,* 2001)
341 and ascribed to local inputs of phosphogypsum, a calcium sulphate that is a by-
342 product of phosphoric acid and naturally enriched with Cd. In mussels, Cd
343 concentrations were highest from the Icelandic sites and lowest in the Seine and
344 Wadden Sea (Table S4). In summary, Cd concentrations were high in sediment, fish
345 and mussels from both Iceland and Cartagena, low in sediment and biota from the
346 Forth; offshore, fish (dab) had elevated Cd concentrations whilst corresponding
347 sediment concentrations were low. Higher concentrations of Cd have previously
348 been reported in cod from Iceland compared to other areas of the North Atlantic
349 (Sturludottir *et al.,* 2014). As Cd has a nutrient-like behaviour, the differences in Cd
350 concentrations in different parts of the region may reflect differences in hydrographic
351 conditions (Yeats, 1988), as well as possible volcanic inputs in Iceland and mining
352 wastes in SE Spain.
353
354 For all matrices, concentrations of Pb (Tables S1-S5; Figs. 3, S2, S4-S6) were
355 highest at Cartagena, exceeding the sediment ERL and the EC food Regulation limit
356 for mussels. As noted above, Cartagena is known to have a high background due to
357 natural geology, and to be contaminated with metals from mining activities.
358 Concentrations of Pb in fish (Table S5; Fig.s S4 and S5) and mussels (Table S4; Fig.
359 S6) from the Forth estuary (Blackness and Alloa sites) were also high compared to
360 other sites, whilst surface sediment concentrations were similar to elsewhere (Fig.
361 S2); this could indicate greater bioavailability of sediment-associated Pb in the Forth,
362 or possibly greater availability from other sources, such as the water column, here
363 than elsewhere. As well as high Pb, flounder from Blackness also had the highest
364 liver Cu and Zn concentrations (Fig. S4 and S5; Table S5; ANOVA, *p*<0.001 for both
365 elements). Increased tissue concentrations of essential metals such as Cu and Zn
366 may be caused by exposure to the metals themselves, but could also be modulated
367 by other stressors. The inner Firth of Forth and the Forth estuary have had over 200
368 years of industrial and domestic inputs (Smout and Stewart, 2012) and the
369 sedimentary reservoir of historic pollutants is therefore significant, particularly in the
370 estuary (Baxter *et al.,* 2011). Elsewhere, sediment Pb concentrations (Fig. S2) were
371 above the ERL at the Dutch Wadden Sea site and the Baltic Sea (Mecklenberg
372 Bight) site, whilst being lowest at the Icelandic sites (1.7-1.9 µg/kg), although only
373 the SE Iceland (IS1) site was assessed as at background for Pb in sediment.
374 Patterns of Pb concentrations in dab liver were similar to those of Cd, with highest
375 values in the central North Sea (especially Ekofisk); however, unlike Cd,

376 concentrations of Pb were low in both sediment and fish liver (both dab and
377 haddock) from Iceland (Tables S1-S5; Figs. S2, S4 and S5).
378
379 High Hg concentrations in sediments, fish and mussels (Tables S1-S5; Fig.s 3, S3-
380 S6) were found at Cartagena, where Hg exceeded the ERL for sediments and the
381 EQS for biota (although not the EC food Regulation limits). Due to underlying
382 geology, much of the Mediterranean Sea has high background levels of Hg
383 (Bernhard, 1988; Martinez-Frias *et al.,* 1998), although the reasons for high biota Hg
384 concentrations in Mediterranean species may also be due to biochemical or
385 ecological factors (Cossa and Coquery, 2005; Cresson *et al.,* 2014). Elsewhere, Hg
386 concentrations exceeded the sediment ERL at most coastal and estuarine sites, and
387 were above background everywhere except the Alde estuary, SE Iceland (IS1) and
388 the central North Sea (Fig. S3). Similarly to Cd and Pb, higher Hg concentrations
389 were found in dab from the central North Sea (where sediment concentrations were
390 low) than in dab from Iceland, the Baltic, or the southern North Sea. With respect to
391 Hg, under European Union Directives, the quality of estuarine, coastal, territorial
392 (Water Framework Directive; WFD) and offshore waters (Marine Strategy
393 Framework Directive; MSFD) in Europe should be assessed using the EQS. This is
394 a legal standard, set for biota and intended to protect against secondary poisoning
395 due to trophic magnification (EC, 2005). Biota Hg concentrations at all sites (except
396 mussels from Hvassahraun and Cape Palos) exceeded the EQS (20 µg kg^{-1} w.w.).
397 The Hg EQS was derived from toxicological data according to the EQS Technical
398 Guidance Document (EC, 2011), but its use presents difficulties as it is set at a level
399 (20 µg/kg w.w.) which is close to or below the natural background of both the
400 OSPAR region (BAC = 18 or 35 µg/kg w.w. for mussels or fish muscle respectively;
401 OSPAR, 2009) and the Spanish Mediterranean (40 or 120 µg/kg w.w. for mussels or
402 fish muscle respectively; Benedicto *et al.,* 2012). Furthermore, the latest EC
403 guidance on the implementation of the biota EQSs states that the EQS is set for
404 animals of Trophic Level 4 and that concentration data should be corrected using
405 trophic magnification factors before comparison with it (EU, 2014). In effect, this
406 reduces the EQS to approximately 4 µg/kg w.w. for mussels or approximately 11
407 µg/kg w.w. for flounder. These values are well below the OSPAR BAC and not
408 attained anywhere in this study, even in relatively remote sites such as Iceland. An
409 important discussion needs to be had around why the toxicological EQS is below
410 background, and of any legal implications for Member States arising from the high
411 frequency of samples/sites exceeding the EQS, but this is beyond the scope of this
412 paper.
413
414
415 3.2.   PCBs
416 PCB concentrations were determined for 8 (of 14) sediment stations.   Three
417 locations (Blackness, Cartagena and Wadden Sea) were assessed as above
418 background (Fig. S7) as they had two or more congeners exceeding their respective
419 BACs, but no sites were assessed as red (≥2 congeners above the EAC). At only
420 Bjarnarhöfn were PCB concentrations in mussels (Table S4) at background (blue),
421 whilst two sites were assessed as red for PCBs in mussels: 2 congeners exceeded
422 EACs at Blackness and 5 congeners did so in mussels from Le Havre in the Seine,
423 where PCB concentrations also exceeded the EC food regulation limit. Dab from the
424 Seine estuary at Parfond also had extremely high liver PCB concentrations
425 (ΣPCB_{ICES-7} = 1668 µg kg^{-1} w.w.; Table S5b), and this was the only site to be

9

assessed as red for PCBs in liver as the concentrations exceeded the EC food regulation limit (fish liver EACs were not used due to a lack of lipid data for normalisation). Our findings of biota PCB concentrations an order of magnitude higher in the Seine than elsewhere is consistent with the Seine Bay and Seine estuary having previously been reported as amongst the most PCB contaminated areas in Europe (Cachot *et al.,* 2006; Bodin *et al.,* 2008). At the other ICON sites, the ΣPCB_{ICES-7} concentrations in fish livers were in the range 10-100 µg kg^{-1} w.w. (Table S4b), which is consistent with reported concentrations in plaice liver from the north-western North Sea (Webster *et al.,* 2011). As with sediments, lowest PCB concentrations in dab liver were recorded at the central North Sea (Egersund Bank, Ekofisk and Dogger Bank; 19-24 µg kg^{-1} w.w.), with relatively high concentrations at the more inshore Baltic Sea and German Bight sites (96-117 µg kg^{-1} w.w.). Whilst absolute sediment PCB concentrations were similar in the German Bight to elsewhere, TOC-normalised sediment concentrations were relatively high (Tables S1 and S2). This probably reflects the transport of fine particles from the River Elbe to the area, whilst the relatively high dab liver concentrations indicate that the sediment PCBs at this site are bioavailable. The River Elbe is a source of contaminants to the North Sea and a pollution gradient from it to the German Bight is well known (Schwarzbauer *et al.,* 2000; Umlauf *et al.,* 2011).

3.3. PAHs

Concentrations of PAHs in sediment (sum of 11 compounds; Fig. 4, Table S1) were extremely variable, ranging from <1 µg/kg d.w. at SE Iceland (IS1), to almost 2,000 µg/kg d.w. at Blackness in the Forth estuary. After normalisation to 2.5% TOC content, concentrations at most sites were in the range 300-350 µg/kg d.w. (Table S2), being slightly lower at Cartagena (182 µg/kg d.w.) and higher at the Seine (539 µg/kg d.w.). Iceland still had very low concentrations (~20 µg/kg d.w.) whilst the Baltic Sea (805 µg/kg d.w.) and Blackness sites (2669 µg/kg d.w.) had high TOC-normalised PAH concentrations. The SE Iceland (IS1), Ekofisk and Egersund Bank sites were assessed as close-to-background for sediment PAHs (<2 compounds above BAC) and all the other sites, except Blackness, were assessed as green; at Blackness all of the PAH compounds were above background in sediment, with phenanthrene and anthracene being above their respective ERLs. Relatively high PAH exposure of mussels was also observed for Blackness compared to the other sites (Table S4), although no PAH exceeded its EAC or EQS. This site is located in the Forth estuary of eastern Scotland, an area that hosts a large oil refinery and petrochemical complex, together with a large coal-fuelled power station and that has been the site of intense industrial activity for over 250 years (Smout and Stewart, 2012). Similar to previous studies (Rocher *et al.*, 2006), high PAH exposure of biota was also observed in this study for mussels at Le Havre (Seine estuary) compared to Le Moulard (Channel coast; Table S4) and for fish at Parfond in the Seine Bay (Kammann *et al.*, this issue), whilst sediment PAH concentrations were also relatively high (173 µg/kg d.w.) in the Seine estuary. PAH concentrations for the Seine in this study were comparable to those of earlier studies. Summing the same compounds (phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene / triphenylene, benzo[*a*]pyrene, benzoperylene, indenopyrene) and converting using a wet/dry weight factor of 5, Rocher *et al*. (2006) reported mussel concentrations of 60 and 2.5 µg/kg w.w. for Le Havre and Le Moulard, whilst here we

476 report 29 and 10 µg/kg w.w. For the same PAH compounds in sediments as
477 reported here, Cachot *et al.* (2006) reported higher concentrations in the Seine
478 estuary (1,500-4,000 µg/kg d.w.) than we do here (173 µg/kg d.w.), but a similar total
479 concentration for Seine Bay sediments (22.5 µg/kg d.w., compared to 39 µg/kg d.w.
480 in this study). High concentrations of PCBs and PAHs in the Seine can be ascribed
481 to a long history of industrial/urban inputs and high atmospheric deposition in the
482 catchment (see also Burgeot *et al.,* this issue).

484 Sediment PAH composition profiles (each PAH as a percentage of the total
485 concentration) were investigated by PCA, both with and without the inclusion of
486 naphthalene (Figs. S8 and S9) as no naphthalene data were available for several
487 sites. In both PCAs, the first two PCs explained 82% of the variation. Coastal North
488 Sea sites (Blackness, Seine estuary, Wadden Sea) separated due to their higher
489 proportions of lighter PAHs, whereas the Baltic Sea, Ekofisk and Egersund Bank
490 sites had progressively greater proportions of heavy PAHs (indenopyrene,
491 benzoperylene and benzofluoranthenes), indicating differing sources of
492 contamination.

494 The sources of PAH contamination can be identified by the concentration-ratios of
495 parent compounds or of alkylated-to parent PAH ratios and the use of parent PAH
496 concentration-ratios was recently reviewed by Tobiszewski and Namieśnik (2012).
497 However, they also caution that environmental factors can influence the behaviour of
498 individual PAH compounds and thus recommend that more than one ratio should be
499 considered in identifying the likely source of PAH contamination. Using the
500 diagnostic anthracene/(anthracene+phenanthrene) ratio (ANT/[ANT+PHEN]) of Pies
501 *et al.* (2008; Table 3) the sediment PAHs in this study would appear to be primarily of
502 pyrolytic origin, except at Parfond (Table 4). The use of the
503 fluoranthene/(fluoranthene+pyrene) ratio (FLUT/(FLUT+PYR); Yunker *et al.,* 2002)
504 suggests that this is mostly due to the combustion of coal and biomass (ratio >0.5),
505 which is generally supported by the use of the
506 indenopyrene/(indenopyrene+benzoperylene) ratio (IcdP/[IcdP+BghiP])which is also
507 >0.5 for most sites, with 0.5 being the ratio distinguishing between petroleum
508 combustion (ratio of 0.2-0.5) and coal or biomass (ratio of >0.5; Yunker *et al.,* 2002).
509 The ANT/(ANT+PHEN) ratio of sediments from Parfond indicates a petrogenic
510 source, although the anthracene concentration was below the detection limits (LoD/2
511 used), and this is not in agreement with the FLUT/(FLUT+PYR) ratio, which indicates
512 a fossil fuel combustion source; benzoperylene was not determined here.
513 Tobiszewski and Namieśnik (2012) indicate that anthracene is one of the PAH
514 compounds more subject to environmental variation and that the ANT/(ANT+PHEN)
515 ratio should not be relied upon for accurate source-apportionment without additional
516 evidence (e.g. ratios measured for known local sources, or other diagnostic ratios in
517 agreement), whereas the FLUT/(FLUT+PYR) ratio is more conservative and thus it
518 seems likely that the source of PAH to Parfond is also pyrolysis of fossil fuels.
519 Sediment PAH at the Blackness site appears to have a petroleum or crude oil
520 combustion source, with FLUT/(FLUT+PYR) and IcdP/(IcdP+BghiP) ratios of 0.45,
521 whilst the ratios do not agree on the source of PAHs at Cartagena or the offshore
522 Firth of Forth.

525 3.4.   Overall assessment

11

526   In the North Sea, the data generally show an increasing gradient of contamination
527   from North to South and from inshore to offshore, which is consistent with other
528   reports (e.g. Salomons *et al.,* 1988; OSPAR, 2010b) and reflects factors such as the
529   higher coastal population density and greater industrial activity.   Contaminant
530   concentrations were generally highest in estuaries (e.g. the Seine, Blackness, the
531   Wadden Sea), at Cartagena in SE Spain (especially metals), and at the Baltic Sea
532   site in the Mecklenberg Bight, whilst being low at the offshore sites and in the Alde
533   estuary of eastern England (Tables 2-4, S2-3). Similarly, the Mecklenberg Bight was
534   found to be of moderate or poor status ("disturbed by hazardous substances") in the
535   most recent HELCOM assessment of hazardous substances (HELCOM, 2010).
536
537   The Icelandic sites stand out has having high concentrations of some metals,
538   particularly Cu and Cd, but low concentrations of organic contaminants such as
539   PCBs and PAHs. Iceland is thought to have low inputs of anthropogenic origin (e.g.
540   Skarphedinsdottir *et al.,* 2010; Kammann *et al.,* this issue) and elevated Cu and Cd
541   concentrations in sediments may reflect a higher degree of mineralisation from
542   volcanic inputs. Higher concentrations of Cd have also been reported in cod from
543   Iceland than from other areas of the North Atlantic (Sturludottir *et al.,* 2014). Higher
544   concentrations of Cd were also observed in fish from offshore than from inshore
545   areas. Furthermore, fish from Ekofisk, offshore Firth of Forth, Dogger Bank, German
546   Bight and the Baltic Sea sites were all approximately 2.5 years old, but had
547   contrasting liver Cd concentrations (generalised as higher in the North and lower in
548   the South). As Cd has a nutrient-like behaviour, the differences in Cd concentrations
549   may reflect differences in hydrographic conditions (Yeats, 1988).   The
550   Mediterranean is known to have high mercury levels (Cossa and Coquery, 2005),
551   whilst the Cartagena area in particular has been subject to historic inputs of metals
552   from mining (Benedicto *et al.,* 2008; 2011) and our findings indicate that sediments
553   and biota (mussels) are still highly contaminated, although direct mining waste inputs
554   ceased in 1990. Concentrations of contaminants were determined in liver from three
555   fish species (dab, flounder and haddock) in northern Europe. Although there was no
556   simple geographical pattern of trace metal concentrations in fish livers, PCB
557   concentrations tended to be highest in livers of fish from the southern North Sea,
558   being particularly high in the Seine Bay and estuary, an area long recognised as
559   being contaminated by PCBs (e.g. Abarnou *et al.,* 1987; Cachot *et al.,* 2006).
560
561   The number and proportion of determinant/matrix combinations assessed in each
562   category for each site are shown in Figure 5. In total, there were 211 possible
563   observations; of these, no data were obtained for 15% of contaminant/matrix
564   combinations (in general this was due to loss of samples/sub-samples), 12% were at
565   background (i.e. $<T_0$), 14% were above the upper (T_1) transition threshold and 44%
566   were between T_0 and T_1. More than 15% of possible observations were above the
567   T_1 transition at the Blackness, Baltic Sea, Wadden Sea, Seine estuary, Le Havre and
568   Cartagena sites.
569
570   The most recent major assessment of contaminants and the environmental status of
571   the North Sea was the OSPAR Quality Status Report (QSR2010; OSPAR, 2010b),
572   which assessed the status and trends of Cd, Hg, Pb, PAH and PCB concentrations
573   in sediment and biota for the period 2003-2007.   Assessments of North Sea
574   concentrations in this study are generally consistent with those of the QSR2010. For
575   some contaminants (e.g. Cd, Pb), concentrations were assessed as of better status

576 in this study than in QSR2010. Assessment of PAH status was similar in this study
577 to in the QSR2010. The three ICON North Sea mussel sites were assessed as
578 green (above BAC, but below EAC) for PAHs, as they were in the QSR2010.
579 Sediment sites common to both studies had the same assessment results for PAHs,
580 with only remote sites at background – here that was at SW Iceland (IS2), whereas
581 in the QSR2010 it was at locations in northern Norway, or the northern North Sea.
582 Assessments of biota PCB concentrations were also similar in this study to in the
583 QSR2010 – with concentrations close to background only in very remote areas (e.g.
584 Iceland here or in northern Norway for the QSR2010). However, ICON sediment
585 PCB assessments generally indicated better status than in the QSR2010.
586 Sediments from the coastal North Sea were mostly red (>EAC) in 2007, but green or
587 blue here, whereas offshore sites were largely green in the QSR2010 and blue here.
588 Slight improvements in the status assessment here compared to in the QSR2010
589 (e.g. Cd and PCBs in sediments) may reflect the different assessment processes. In
590 the QSR, OSPAR compared the BAC/EAC with the error around the fitted value from
591 the last year of a trend assessment, whereas here we compared either the
592 mean±95% confidence interval or the measurement uncertainty with the assessment
593 criteria. Sediment mercury concentrations were above the ERL in most coastal
594 North Sea sediments in both this study and the QSR2010, with biota concentrations
595 very rarely at background in either study. In this study, the biota assessment for Hg
596 was usually red (above the EQS), whereas in the QSR2010 biota were usually
597 assessed as green as OSPAR used the EC food regulation for the upper transition,
598 rather than the EQS; the food regulation limit was not exceeded in this study.
599
600 In summary, the Icelandic sites had relatively low concentrations of organics and
601 most metals, but relatively high concentrations of Cd and Cu, possibly due to
602 volcanic inputs. The offshore North Sea sites generally had relatively low
603 concentrations of contaminants, although fish in some areas had relatively high liver
604 Cd concentrations, possibly related to hydrographic conditions. The inshore and
605 estuarine North Sea sites had the highest contaminant concentrations, particularly
606 the Seine (PCBs and PAHs), Forth estuary (PAHs and Hg) and Wadden Sea
607 (PCBs/metals). The Baltic Sea site (Mecklenberg Bight) had relatively high
608 concentrations of lead and PAHs in sediments, and of PCBs in flounder (but not in
609 dab) livers. The SE of Spain had relatively high metals concentrations, ascribed to
610 local inputs from mining wastes and natural geology. At many sites, upper-level
611 assessment criteria were exceeded, and at no sites were concentrations of all
612 substances at background. These findings reflect earlier studies and indicate a need
613 to investigate whether adverse biological effects are occurring as a result of
614 simultaneous exposure to multiple contaminants, and a need to produce a holistic
615 assessment of environmental health at these sites, based upon chemical
616 concentrations and concomitant biological responses. These needs were also
617 investigated during the ICON project and are presented in the accompanying papers
618 of this issue (e.g. Burgeot *et al.,* Carney Almroth *et al.,* Hylland *et al.,* Kammann *et*
619 *al.,* Lang *et al.,* Lyons *et al.,* Martínez-Gómez *et al.,* Vethaak *et al.,* all this issue). A
620 full assessment of the ICON data, including effects, can be found elsewhere in this
621 issue (Hylland et al., this issue).
622
623
624 4. Conclusions

This study shows that exposure of marine organisms to so-called legacy environmental contaminants continues to be a pan-European issue, particularly for industrialised estuaries and nearby coastal waters. The study further highlights the benefits of the traffic-light approach to assessing the environmental status of contaminants as it readily summarises the significant findings, enabling communication with policymakers, stakeholders and the general public. The wide geographic scale of this study also indicates the suitability of the approach for conducting the (sub-) regional sea scale assessments that are required under the Marine Strategy Framework Directive. There remains a need to know whether multiple contaminant exposures compromise environmental health and this requires an integration of knowledge on both contaminant concentrations and their biological impacts.

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940   7. Supplementary Information
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942   Figure S1: Sediment Cd concentrations (mg/kg dry weight, normalised to 5%
943   aluminium).
944   Figure S2: Sediment Pb concentrations (mg/kg dry weight), normalised to 5%
945   aluminium.
946   Figure S3: Sediment Hg concentrations (µg/kg dry weight, normalised to 5%
947   aluminium).
948   Figure S4: Concentrations of trace metals (mg/kg wet weight) in fish liver
949   Figure S5: Principle Components Analysis score plot of trace metal
950   concentrations in fish liver
951   Figure S6: Principle Components Analysis score plot of trace metal
952   concentrations in mussels
953   Figure S7: Sediment PCB concentrations (sum of CBs 28, 52, 101, 118, 153,
954   180; µg/kg dry weight, normalised to 2.5% total organic carbon).
955   Figure S8: Principle Components Analysis score plot for the composition of
956   Polycyclic Aromatic Hydrocarbons (including naphthalene) in
957   sediments
958   Figure S9: Principle Components Analysis score plot for the composition of
959   Polycyclic Aromatic Hydrocarbons (excluding naphthalene) in
960   sediments
961

962   Table S1: Sediment contaminant concentrations and physicochemical
963   characteristics.
964   Table S2: Sediment contaminant concentrations normalised to 5% Al (metals)
965   or 2.5% TOC (organics; µg/kg d.w.).
966   Table S3: Sediment trace metal concentrations (mg/kg dry wt), normalised to
967   100% fine grained (<63 µm) material.
968   Table S4: Contaminant concentrations (µg/kg wet weight) in mussels
969   Table S5: Fish age and concentrations (µg/kg wet weight) of contaminants in
970   (a) fish muscle and (b) fish liver
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973 Figure 1: Sampling locations for sediment and mussels (A), or fish (B). For
974 explanation of site codes, see Table 1.
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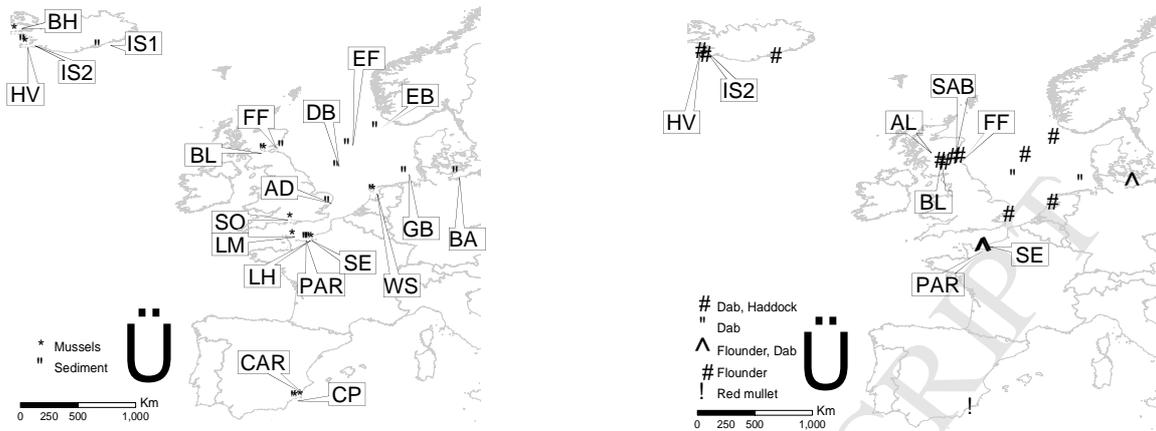
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979 Figure 2: Assessment of data using the "traffic light" system (amended from
980 OSPAR, 2008 and UNEP/MAP, 2011). A = concentrations below the lower transition
981 point (T_0) and at background; B & C = above background, but not of environmental
982 concern; D & E = above the upper transition level (T_1) and adverse impacts possible.
983 EQS = EU Environmental Quality Standard; EAC = Environmental Assessment
984 Criteria; ERL = US Effects Range Low; MPC = Maximum Permitted Concentration
985 under EC food Regulations; BAC = Background Assessment Concentration. In
986 OSPAR (2008) concentrations between the BAC and the food regulation limit were
987 coloured amber, rather than green.

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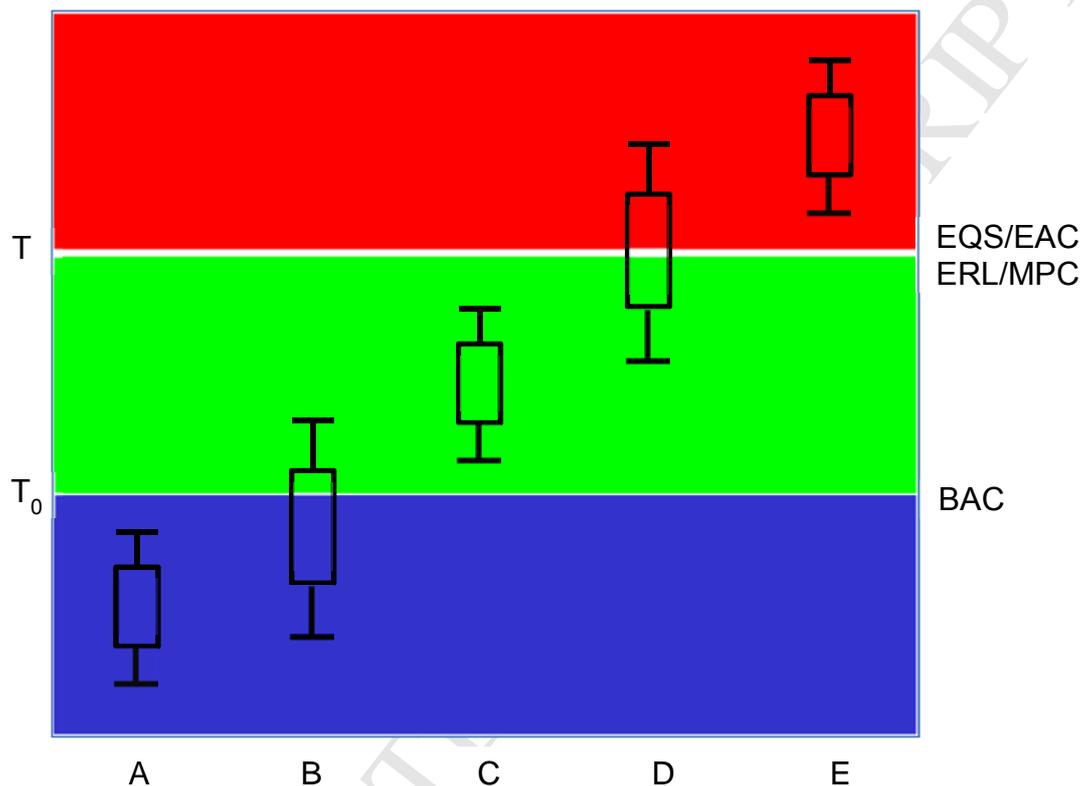
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991   Figure 3:   Principle Components plot of sediment trace metals concentrations
992   (normalised to 5% aluminium). PC1 = high metal concentrations (67% of total
993   variation); PC2= very high Cu and higher Cd concentrations (17% of total variation).
994   Site code abbreviations defined in Table 1.
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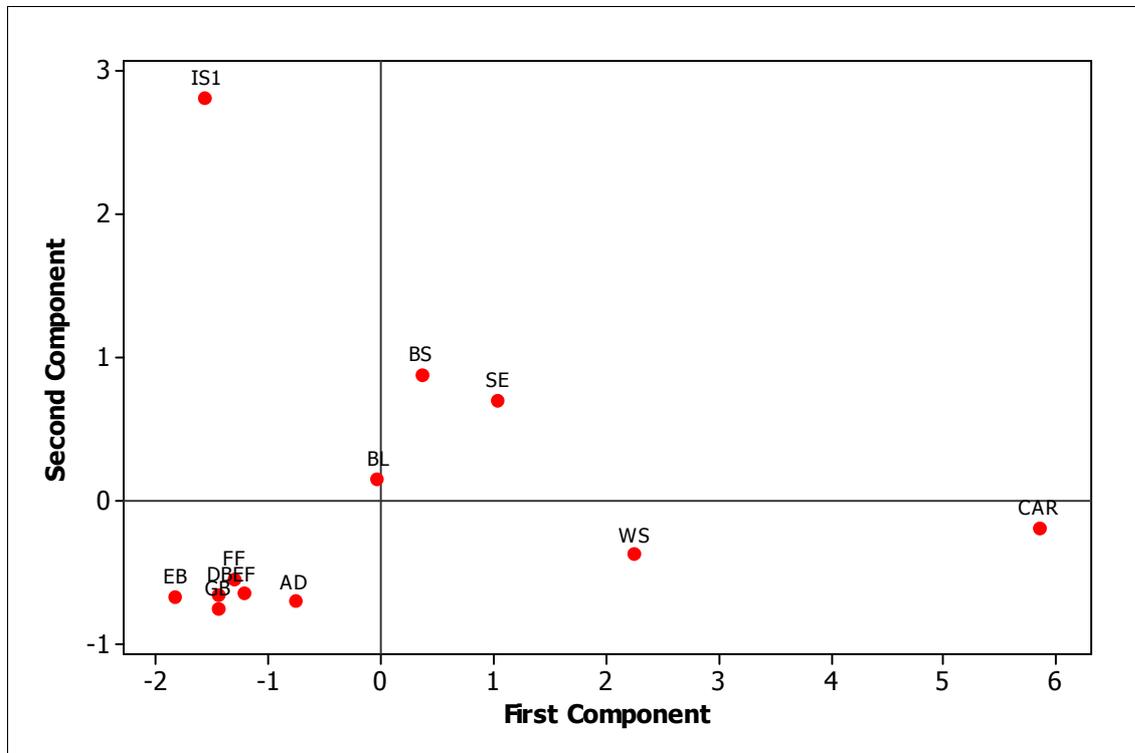
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1001 Figure 4: Sediment PAH concentrations (sum of naphthalene, phenanthrene,
1002 anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene/triphenylene,
1003 benzofluoranthenes, benzo[*a*]pyrene, indenopyrene, benzoperylene; µg/kg dry
1004 weight normalised to 2.5% total organic carbon). Colours indicate the status
1005 assessment, which is based upon the number of individual compounds exceeding
1006 their respective assessment criteria, rather than total PAH concentration; blue =
1007 concentrations at background, green = concentrations of 2 or more individual PAHs
1008 elevated (>BAC), but not of significant environmental concern, red = concentrations
1009 elevated for 2 or more individual PAHs (>ERL) and could cause adverse effects.
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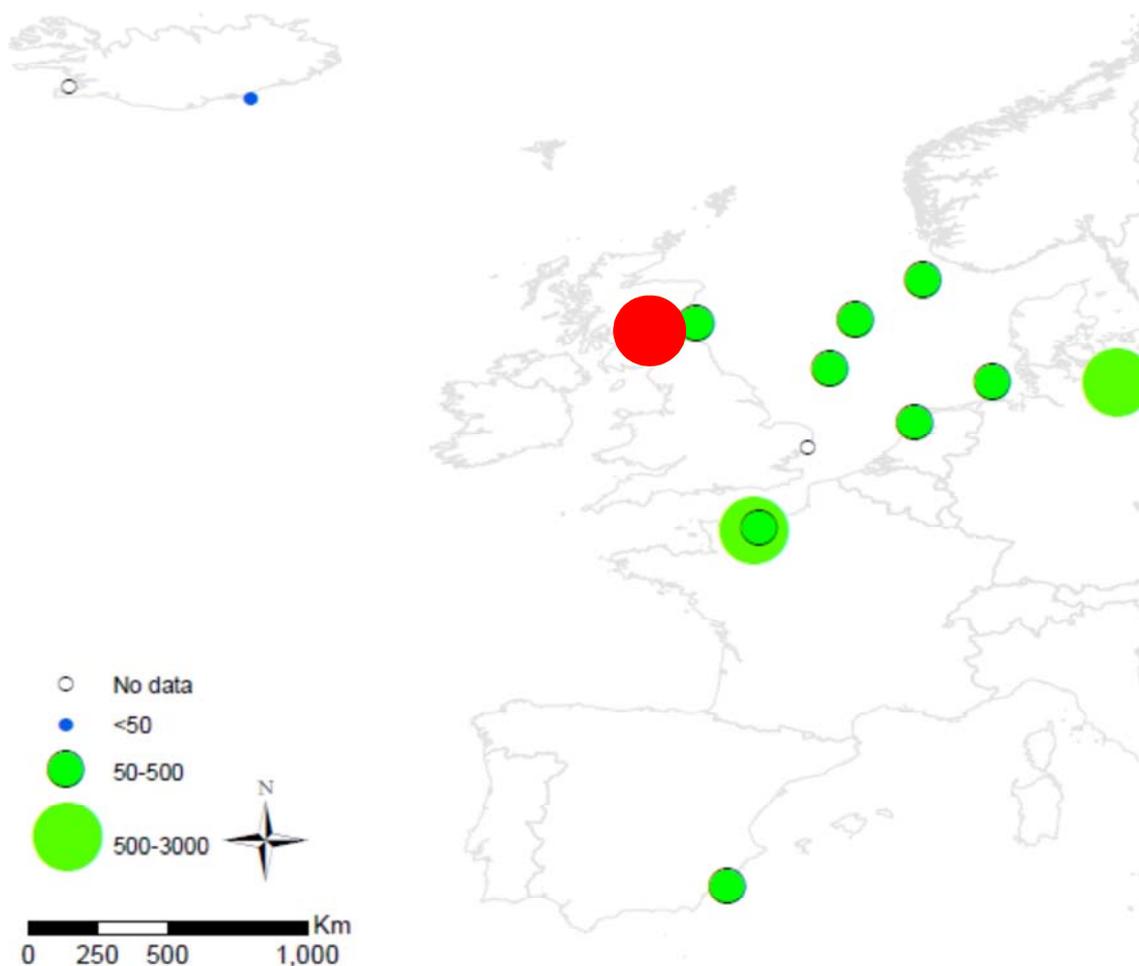
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1013 Figure 5: Number (a) and proportion (b) of results in each assessment category.
1014 Blue = concentrations close-to- background (<T0), green = concentrations elevated,
1015 but not of significant environmental concern, red = concentrations elevated and could
1016 cause adverse effects (>T1); grey = no data.
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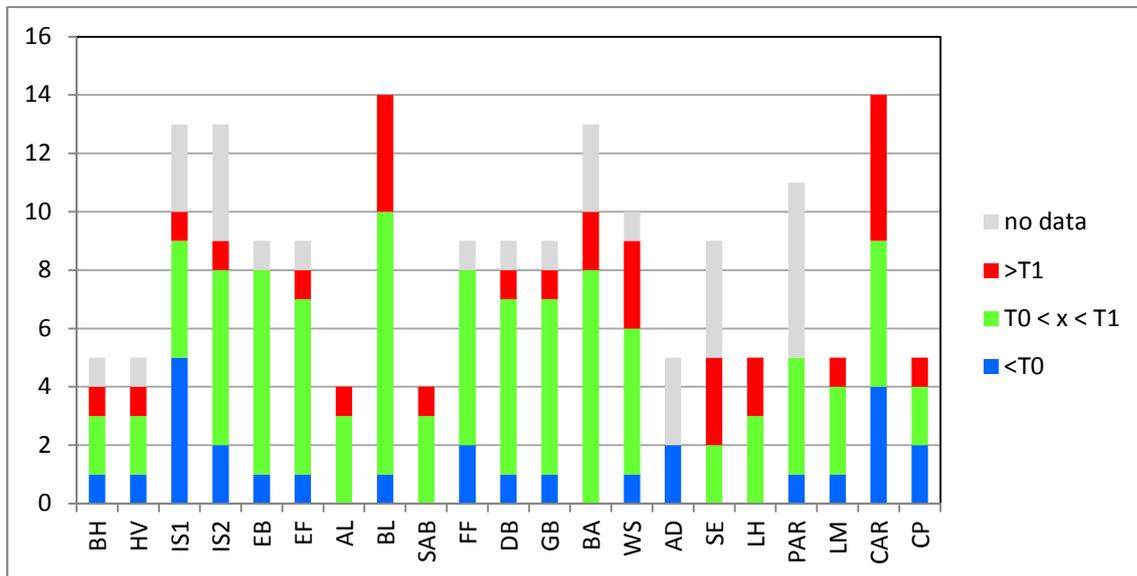
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1021   Table 1: Sampling site information.
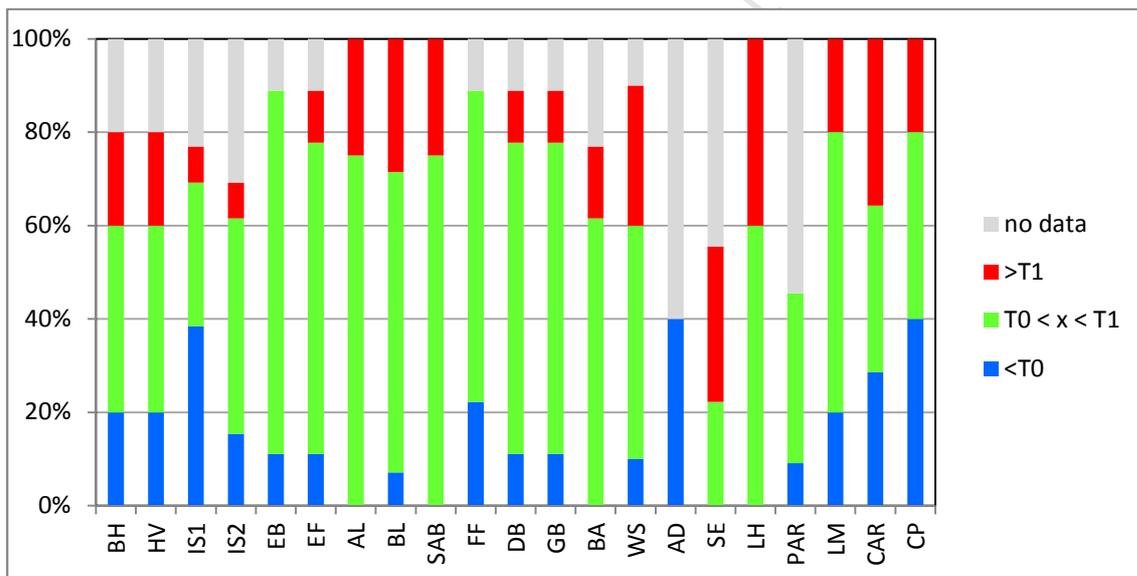1022

Region	Site name	Site code	Site type	Latitude	Longitude	Samples collected
Iceland	Bjarnarhöfn	BH	Coastal	65.000	-22.970	Mussels
Iceland	Hvassahraun	HV	Coastal	64.023	-22.146	Mussels
Iceland	SE Iceland	IS1	Offshore	63.767	-16.404	Dab, haddock, sediment
Iceland	SW Iceland	IS2	Offshore	64.146	-22.280	Dab, haddock, sediment
North Sea	Egersund bank	EB	Offshore	57.708	5.295	Haddock, sediment
North Sea	Ekofisk	EF	Offshore	56.402	3.095	Dab, haddock, sediment
North Sea	Forth estuary - Alloa	AL	Estuary	56.110	-3.810	Flounder
North Sea	Forth estuary - Blackness	BL	Estuary	56.014	-3.480	Flounder, mussels, sediment,
North Sea	Firth of Forth - St Andrews Bay	SAB	Coastal	56.407	-2.739	Flounder
North Sea	Firth of Forth - offshore	FF	Offshore	56.300	-2.034	Dab, haddock, sediment
North Sea	Dogger Bank	DB	Offshore	54.748	2.271	Dab, sediment
North Sea	German Bight	GB	Offshore	54.338	7.537	Dab, sediment
Baltic Sea	Mecklenberg Bight, Baltic Sea	BA	Coastal	54.333	11.600	Flounder, dab, sediment
North Sea	Wadden Sea	WS	Coastal	52.965	5.017	Mussels, sediment
North Sea	Alde	AD	Estuary	52.096	1.561	Sediment
English Channel	Seine estuary	SE	Estuary	49.431	0.003	Dab, sediment
English Channel	Le Havre	LH	Estuary	49.423	0.209	Mussels
English Channel	Parfond (Seine Bay)	PAR	Coastal	49.385	-0.154	Dab, flounder, sediment
English Channel	Le Moulard	LM	Coastal	49.653	-1.238	Mussels
Mediterranean Sea	Cartagena	CAR	Coastal	37.562	-1.030	Red mullet, mussels, sediment
Mediterranean Sea	Marine Reserve of Cape Palos	CP	Coastal	37.653	-0.653	Mussels

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1025 Table 2: Criteria used in assessing ICON contaminant data. Values are dry weight for sediments (with OSPAR BACs normalised
1026 to 5% Al or 2.5% TOC[2]), wet weight for biota (BACs/EACs converted from dry weight using a factor of 5). BAC = Background
1027 Assessment Concentration; EAC = Environmental Assessment Criteria; ERL = Effects Range Low; EQS = Environmental Quality
1028 Standard; MPC = Maximum Permitted Concentration. BACs were used for the blue/green assessment transition (T_0); EQSs,
1029 EACs, ERLs or MPCs were used for the green/red assessment transition (T_1).
1030

	Sediment			Mussels				Fish liver		Fish muscle			
Metals (mg/kg)	BAC Spain[1]	BAC[2]	ERL[3]	BAC Spain[4]	BAC[2]	EQS[5]	MPC[6]	BAC[2]	MPC[6]	BAC Spain[4]	BAC[2]	EQS[5]	MPC[6]
Cd	0.15	0.31*	1.2	0.22	0.19		1	0.026	1	0.002	0.026		0.05
Hg	0.045	0.07*	0.15	0.04	0.018	0.02				0.12	0.035	0.02	
Pb	30	38*	46.7	0.76	0.26		1.5	0.026	1.5	0.11	0.026		0.3

	Sediment			Mussels			
PAHs (µg/kg)	BAC Spain[2]	BAC[2]	ERL[3]	BAC Spain[4]	BAC[2]	EAC[2]	EQS[5]
Naphthalene		8	160			68	
Phenanthrene	7.3	32**	240	4.86	2.2	340	
Anthracene	1.8	5**	85	0.82		58	
Fluoranthene	14.4	39**	600	1.36	2.44		30
Pyrene	11.3	24**	665	1.22	1.8	20	
Chrysene	8	20**	384	0.42	1.62		
Benz[a]anthracene	7.1	16**	261	0.26	0.5	16	
Benzo[k]fluoranthene				0.36		52	
Benzo[a]pyrene	8.2	30**	430	0.26	0.28		5
Benzo[g,h,i]perylene	6.9	80**		0.26	0.5	22	
Indeno[1,2,3-c,d]pyrene	8.3	103**		0.16	0.48		

	Sediment				Mussels			Fish liver		Fish muscle	
PCBs (µg/kg)	BAC Spain[2]	ERL[3]†	BAC[2]	EAC[2]	BAC Spain[2]	BAC[2]	EAC[2]	BAC[2]	MPC[6]	BAC Spain[2]	MPC[6]
CB 118			0.17	0.6**	0.024	0.12	0.24	0.12	-	0.02	-
CB 28			0.22	1.7**	0.03	0.15	0.64	0.15		0.02	
CB 52			0.12	2.7**	0.03	0.15	1.08	0.15		0.016	
CB 101	0.46†	11.5†	0.14	3**	0.028	0.14	1.2	0.14	200††	0.016	
CB 138			0.15	7.9**	0.024	0.12	3.16	0.12		0.018	75††
CB 153			0.19	40**	0.024	0.12	16	0.12		0.02	
CB 180			0.1	12**	0.024	0.12	4.8	0.12		0.022	

1031 [1] UNEP/MAP, 2011; [2] OSPAR, 2009; [3] NOAA, 1999; [4] Benedicto *et al.,* 2012; [5] Priority Standards Directive (2013/39/EU); [6] EU Food Regulation (EC/1881/2006 as amended)
1032 *Normalised to 5% Al; **normalised to 2.5% TOC; †Sum of 7 indicator PCBs (used for sediment in SE Spain); ††Sum of 6 indicator PCBs (CB 118 excluded)

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1033 Table 3: Polycyclic aromatic hydrocarbon (PAH) ratios used for source apportionment in this study (reviewed in Tobiszewski and
1034 Namieśnik, 2012).
1035

Ratio	Value	Source	Citation
Anthracene / (anthracene + phenanthrene)	<0.1	Petrogenic	Pies *et al.*, 2008
	>0.1	Pyrolytic	
Fluoranthene / (fluoranthene + pyrene)	<0.4	Petrogenic	Yunker *et al.*, 2002
	0.4-0.5	Vehicle and crude oil combustion	
	>0.5	Coal / biomass combustion	
Indeno[1,2,3,*c,d*]pyrene / (indeno[1,2,3,*c,d*]pyrene + benzo[*g,h,i*]perylene)	<0.2	Petrogenic	Yunker *et al.*, 2002
	0.2-0.5	Vehicle and crude oil combustion	
	>0.5	Coal / biomass combustion	

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1038 Table 4: Polycyclic aromatic hydrocarbon (PAH) source diagnostic ratios observed for sediments in this study. ANT = anthracene;
1039 PHEN = phenanthrene; FLUT = fluoranthene; PYR = pyrene; IcdP = indeno[1,2,3,*c,d*]pyrene; BghiP = benzo[*g,h,l*]perylene. <DL=
1040 below detection limit; n.d. = no data
1041

	IS1	IS2	EB	EF	BA	BL	FF	DB	GB	PAR	SE	WS	AD	CAR
ANT / (ANT+PHEN)	<DL	n.d.	0.20	0.14	0.18	0.27	0.49	<DL	0.13	0.01	0.25	0.22	n.d.	0.19
FLUT / (FLUT+PYR)	<DL	n.d.	0.58	0.57	0.53	0.45	0.41	<DL	0.55	0.55	0.55	0.58	n.d.	0.57
IcdP / (IcdP + BghiP)	<DL	n.d.	0.55	0.54	0.55	0.45	0.56	<DL	0.55	n.d.	0.54	0.53	n.d.	0.45

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