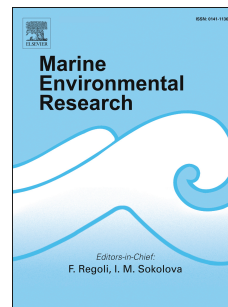


Accepted Manuscript

Comparison of caged and native blue mussels (*Mytilus edulis* spp.) for environmental monitoring of PAH, PCB and trace metals

Merete Schøyen, Ian J. Allan, Anders Ruus, Jarle Håvardstun, Dag Ø. Hjermann, Jonny Beyer



PII: S0141-1136(17)30267-2

DOI: [10.1016/j.marenvres.2017.07.025](https://doi.org/10.1016/j.marenvres.2017.07.025)

Reference: MERE 4357

To appear in: *Marine Environmental Research*

Received Date: 20 April 2017

Revised Date: 28 July 2017

Accepted Date: 31 July 2017

Please cite this article as: Schøyen, M., Allan, I.J., Ruus, A., Håvardstun, J., Hjermann, Dag.Ø., Beyer, J., Comparison of caged and native blue mussels (*Mytilus edulis* spp.) for environmental monitoring of PAH, PCB and trace metals, *Marine Environmental Research* (2017), doi: 10.1016/j.marenvres.2017.07.025.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 Comparison of caged and native blue mussels (*Mytilus edulis* spp.) for 2 environmental monitoring of PAH, PCB and trace metals

3 Merete Schøyen^a, Ian J. Allan^a, Anders Ruus^{a,b}, Jarle Håvardstun^a, Dag Ø. Hjermann^a,
4 Jonny Beyer^{a,*}

5 ^a Norwegian Institute for Water Research (NIVA), Gaustadalléen 21, NO-0349 Oslo, Norway

6 ^b University of Oslo, Department of Biosciences, NO-0316 Oslo, Norway

7 *Corresponding author: Tel.: (+ 47) 98215431, e-mail address: JOB@niva.no (Jonny Beyer)

8 Abstract

9 Contaminant bioaccumulation was studied in blue mussels (*Mytilus edulis* spp.) using the harbor
10 waters of Kristiansand (Norway) as a case study. A suite of chemical contaminants (trace metals,
11 PAHs and PCBs) was analyzed in caged and native mussels as well as in passive samplers (Diffusive
12 Gradients in Thin films (DGT)-devices and silicone rubbers) placed alongside the mussels for
13 estimation of contaminant concentrations in water and uptake rates and bioaccumulation factors
14 (BAFs) in mussels during a six-months deployment period. Estimated logBAFs were in the ranges 2.3
15 - 5.5, 3.8 - 5.2 and 3.2 - 4.4 for metals, PCBs and PAHs, respectively. Contaminant levels in caged
16 mussels increased rapidly to stable levels for trace metals, whereas for hydrophobic organic
17 contaminants the increase was steady but slow and for many compounds did not reach the levels
18 observed in native mussels. Some key issues related to mussel caging design, such as mussel
19 deployment time and confounding influence from seasonal fluctuations, are discussed herein.

20 Keywords: blue mussels; biomonitoring; caging; contaminant bioaccumulation factors

22 1. Introduction

23 Blue mussels (*Mytilus* spp.) are widely used as sentinels in coastal pollution monitoring (mussel
24 watch) programs, mainly because their biological characteristics make them very suitable as
25 bioindicators for assessing the quality status of coastal waters (Farrington et al., 2016; Beyer et al., this
26 volume). Most often mussel watch studies involve collection of samples from natural blue mussel
27 populations, but the adoption of an active biomonitoring alternative by using transplanted blue mussel
28 has gained considerable popularity in ecotoxicology research and monitoring. Indeed, the
29 straightforwardness of using controlled deployments is one of the key advantages with blue mussels in

30 marine monitoring. The comparability of deployed and native mussels in pollution biomonitoring has
31 therefore been investigated in a number of field studies, e.g. (Regoli and Principato, 1995; Peven et al.,
32 1996; Walsh and O'Halloran, 1998; Nasci et al., 2002; Ericson et al., 2002; Nigro et al., 2006), and
33 others have suggested that an integrated use of monitoring data from both native and transplanted
34 mussels may provide a more accurate assessment of pollutant uptake and effect phenomena at
35 contaminated field locations, e.g. (Bodin et al., 2004; Bebianno et al., 2007; Serafim et al., 2011;
36 Brooks et al., 2012). A key question for all such caging studies is how long the blue mussels should
37 stay deployed to be fully representative as a biological sample for assessment of pollutant
38 concentrations and other ecotoxicological parameters.

39 Mussel caging is particularly useful when indigenous mussels are scarce or absent at the planned study
40 sites. The mussel caging alternative is therefore increasingly more being used in trend monitoring
41 (spatial and temporal) and in industrial compliance monitoring (e.g. comparing to quality standards or
42 regulatory benchmarks). However, the actual comparability of caged and native mussels under the
43 specific study conditions is often insufficiently documented. It may for example be relevant to clarify
44 how key factors such as deployment time, caging design (e.g. fixed or floating mussel rig setups),
45 genetic homogeneity/variability of the caged mussels, etc., could affect the general outcome of the
46 study. The investigator may often want to manipulate key study factors (such as timing and duration
47 of exposure, positioning of the caged specimens, etc.) in a controlled manner to create more accurate
48 study designs and to increase the overall quality of the monitoring data. In Norway, technical
49 requirements for mussel monitoring are embedded in national environmental regulations which
50 recently were updated to comply with demands expressed in the EU Water Framework Directive
51 (WFD, 2000/60/EC) and the Marine Strategy Framework Directive (MSFD, 2008/56/EC). These are
52 two wide-ranging trans-national environmental legislation frameworks designed for the protection and
53 restoration of aquatic environments in Europe, see Borja et al. (2010). Relevant requirements relate to
54 representative positioning of stations, choice of sample matrices and the use of quality standards (QSSs)
55 for evaluation of quality status based on contaminant concentration data. With this in mind, a further
56 harmonization of the concept of mussel caging could be important, as it may facilitate the
57 standardization of field monitoring designs and better comparability of coastal monitoring conducted
58 in different countries.

59 In this study, we study contaminant bioaccumulation in blue mussels transplanted to the waters of the
60 city harbor of Kristiansand (Norway), an area known to be moderately to severely polluted by a
61 mixture of inorganic and organic contaminants; especially nickel, copper, cobalt, polycyclic aromatic
62 hydrocarbons (PAHs) and hexachlorobenzene (HCB). The caged mussels, and also native mussels
63 from the harbor, were repeatedly sampled during a period of six months and analyzed for trace metals,
64 polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and chlorinated

65 benzenes. Co-deployed passive sampling devices (Diffusive Gradients in Thin-films (DGTs) and
66 silicone rubbers) were used to estimate freely dissolved contaminant concentrations in the seawater
67 and this allowed the calculation of uptake and excretion rates as well as bioaccumulation factors
68 (BAFs) of contaminants in mussels based on first-order single-compartment toxicokinetics. The results
69 of the present study are relevant in the context of an ongoing work coordinated by the Norwegian
70 Environmental Agency (NEA) and Standard Norway (SN) aiming to develop a Norwegian Standard
71 (NS) for how to use blue mussels and blue mussel caging in marine pollution monitoring.

72 **2. Material and methods**

73 **2.1 Study design and field work**

74 The field work was conducted in the period late May – late November 2015. A suitable number of
75 blue mussels (size range 3-5 cm) was obtained from a mussel farm located in Kaldvellfjord (Lillesand,
76 Norway), a locality distant from known point sources of contamination. First, two replicate samples
77 each including 60 individual mussels were grouped and frozen to serve as before-deployment controls
78 for the caged mussels. The other mussels were transported rapidly (in a cooling box equipped with
79 cooling elements and some brown algae to keep humid conditions) to the caging stations in the
80 Kristiansand city harbor area. The mussels were out of water for only about two hours. Caging
81 Stations 1 and 2 were located in the inner and outer part of the harbor, respectively (Figure 1). Station
82 1 (GPS position 58.13713, 7.97239) was located by the quay of a metal processing plant that produces
83 high quality Ni as well as Co and Cu, whereas Station 2 was located about 2 km in SSE direction from
84 Station 1 in the outer harbor area by the small islet Svensholmen (58.12546, 7.9878).

85 Mussel caging rigs, based on collapsible 5-floor lantern nets (1 m vertical height), were prepared and
86 equipped with approx. 1000 mussels per rig. Passive samplers (DGTs and silicone rubbers) were also
87 mounted in duplicate at each rig. Field control samplers were used to assess contamination in
88 unexposed samplers and in the case of silicone rubber samplers to measure initial performance
89 reference compound concentrations. Samplers were all from the same batches and were all analyzed
90 together. The rigs were positioned in the sea by means of buoys, ropes and weights at each station.
91 The upper end of the lantern net was approx. at 2 m depth. Native mussels of suitable size were only
92 living at caging Station 2 (Svensholmen). The mussel population at Station 2 had been monitored
93 annually since 1998 in connection with the Norwegian contribution to the Oslo-Paris Commission
94 (OSPAR) Joint Assessment and Monitoring programme (JAMP) (Green et al., 2016). After the start of
95 mussel deployment at May 29th, 2015 (day 0) each rig was sampled after approximately one month
96 (July 2nd, 34 days), three months (Sept. 10, 104 days) and six months (Nov. 26, 181 days). Samples of
97 native mussels (at Station 2, Svensholmen) were collected at the start-up day (in late May) and

98 subsequently the same days as for the caged mussels. For each sampling day, approx. 150 mussels
99 were retrieved from each rig and from the Svensholmen population and transported (cold and humid)
100 to the NIVA laboratory (Grimstad) to be frozen and stored to sample preparation. The mussels were
101 not depurated before freezing. Sea temperature data at caging stations were obtained at each sampling
102 day. Unfortunately, at the last sampling, the mussel rig at Station 2 had disappeared for an unknown
103 reason, thus these data (caged mussels and passive samples after six months at Station 2) are lacking in
104 this study. The DGT samplers at both rigs were retrieved after one month of deployment to limit the
105 impact of fouling developing at the surface of the sampler.

106 **2.2 Sample preparation and chemical analyses**

107 The mussel sample preparation was performed according to the OSPAR guidelines (OSPARCOM,
108 2012) and with further details described by Green et al. (2016). The frozen mussels were thawed and
109 two replicate composite samples (each consisting of 60 mussels) per station and per sampling day
110 were prepared for the transplanted and native mussels, respectively. The number of composite samples
111 was decided based on cost-effectiveness. In general, the optimal number of composite samples and the
112 number of individuals per sample depends on the cost of chemical analyses relative to sampling and
113 sample preparation, as well as the level of inherent variation among individuals due to e.g.
114 physiological factors (Bignert et al., 2014). The shells were scraped clean on the outside; the length
115 was measured by means of slide calipers; all soft tissue was scraped out by using a scalpel, weighed,
116 and merged to a composite sample which was weighed before it was frozen and stored at -20 °C until
117 further homogenization and analysis. Empty shells were dried and then weighed sample-wise for
118 condition index estimation. Each pooled mussel sample was analyzed for As, Cd, Cr, Cu, Hg, Ni, Pb,
119 Zn, the 16 US EPA PAHs (EPA Methods 550.1/610/8100/8270C/8310), the 7 indicator PCBs (CB 28,
120 52, 101, 118, 138, 153 and 180), hexachlorobenzene, pentachlorobenzene, fats/lipids and dry matter
121 by using the analytical methods described by Green et al. (2008). Quality Assurance (QA) of chemical
122 analyses at NIVA and Eurofins are carried out by participation in international intercalibration
123 exercises (QUASIMEME) and other relevant proficiency testing programs with acceptable results
124 (Green et al., 2016). Certified reference materials (CRM), Standard Reference Materials (SRM) (e.g.
125 DORM-4 fish protein and QUASIMEME reference biota samples) and in-house reference materials
126 are analyzed routinely. The laboratories are accredited according to ISO/IEC 17025:2005. Chemical
127 analyses were performed on wet tissue samples and the content of solids and lipid were measured to
128 enable statistical examination of chemical concentration data at a wet weight (wet wt.), dry wt., and
129 lipid wt. basis. The data reporting format is specified in table and figure legends. Freely dissolved
130 contaminants concentrations (C_{free}) were estimated from passive samplers, DGTs for metals and
131 silicone rubbers for PCBs and PAHs. The DGT passive samplers were analyzed for Al, Ca, Cd, Co,
132 Cr, Cu, Fe, Ni, Pb and Zn (but not Hg), while the silicone rubber samplers were analyzed for the 16

133 US EPA PAHs, the 7 indicator PCBs, HCB and pentachlorobenzene following procedures described
 134 by Allan et al. (2013). The preparation, extraction and analysis procedures and data of DGT and
 135 silicone rubber passive samplers are shown in the Supporting Information. Field control passive
 136 samplers were used to estimate possible contaminant levels present in non-exposed samplers and in
 137 the case of silicone rubber passive samplers, the measurement of initial performance reference
 138 compound concentrations, as recommended by Booij et al. (2006) for silicone rubber and by Dabrin et
 139 al. (2016) for DGT passive samplers. The NIVA laboratory participated in QUASIMEME
 140 intercomparison exercises on passive sampling with AlteSil™ silicone rubber in 2014 and 2015 and
 141 obtained excellent results.

142 2.3 Treatment and statistical examination of data

143 Contaminant concentrations in caged and native mussels and in passive samplers were compared and
 144 examined for station-wise and temporal trends during the six months' study period. Single-
 145 compartment uptake/elimination modelling was employed for evaluating the contaminant
 146 bioaccumulation processes. Statistical analysis was performed with the use of R software (version
 147 3.3.2) and Statistica software (version 7.1, StatSoft, Tulsa, OK, USA). Differences were evaluated
 148 using Analysis of Variance (ANOVA). The small sample size is itself not invalidating ANOVA so
 149 long as the assumptions are met. Checking the normality assumption, which is critical with a low
 150 sample size, is not feasible using graphical methods as there are only two samples per
 151 site/date/treatment. However, the use of composite samples of a large number of mussels has the effect
 152 of normalizing the data greatly. Thus, even when the distribution of concentrations in individual
 153 mussels is extremely skewed, the distribution of concentrations in a composite sample of 60 mussels is
 154 expected to be close to normal. Levene's test was used to test for heterogeneity of variance. When
 155 necessary, data were \log_{10} -transformed to reduce heterogeneity of variance. In some cases, both
 156 deployed and native mussels showed the same general and approximately linear trends over time; in
 157 these cases, we used ANCOVA to analyze the difference between deployed and native mussels across
 158 sampling occasions. A significance level of $\alpha = 0.05$ was chosen. Due to the low sample size, p-values
 159 should be interpreted with some caution when p-values are between 0.01 and 0.05; on the other hand,
 160 it should also be kept in mind that the statistical power is low. The regression tool in Sigmaplot was
 161 used to obtain BAFs and contaminant depuration rates (k_2) for contaminants accumulating in mussels.
 162 Modelling of the uptake of organic contaminants (PAHs and PCBs) in native mussels at the
 163 Svensholmen site was done by using Equation (1), as described by Björk and Gilek (1999):

$$164 \quad C_{m,t} = C_{m,t_0} + C_{free} \left(BAF - \frac{C_{m,t_0}}{C_{free}} \right) (1 - e^{-k_2 t}) \quad (\text{Eq. 1})$$

165 where C_m is the concentration in mussels (ng/kg wet wt.), C_{free} is the freely dissolved concentration
 166 from silicone rubbers (ng/L), BAF is in L/kg, and k_2 is the 1st order mussel depuration rate (d^{-1}). C_{m,t_0}

167 and C_{free} are known, BAF and k_2 were estimated from the modelling of contaminant uptake at Station
168 1 (industrial harbor site) (when k_2 values were not obtained, we used the median of values reported for
169 PAHs or PCBs), and t is either 34 or 104 d. The use of Equation 1 in relation to data obtained from
170 passive samplers was performed as described by Booij et al. (2006).

171 3. Results

172 Biological data and the results of chemical contaminant measurements in 0-group reference, deployed
173 and native mussels in the present study are shown in Table 1. The survival of deployed mussels during
174 the six-months caging period was very good, with practically no mortality, but during the caging
175 period a lowering trend of lipid content was recorded in caged mussels. This trend was also observed
176 in native mussels, indicating seasonal fluctuations in the study area. However, this general decrease of
177 lipid content would obviously have an influence on the accumulation of contaminants, and in
178 particular of the hydrophobic substances.

179 The chemical analysis of the reference mussels from the donor site (Kaldvellfjord) confirmed
180 generally low contaminant concentrations in the pre-deployed mussels (Table 1), except for Cu which
181 was found to be approximately twice the level expected for an unpolluted sample. After being
182 transplanted, a substantial increase of multiple contaminants was observed in caged mussels at both
183 stations. The increase was most pronounced for nickel at Station 1 (Figure 2), which increased up to
184 35 times when compared with the concentration in pre-deployed mussels (Table 1). The DGT passive
185 sampler accumulates labile metal species from solution while deployed *in situ*, thereby providing an
186 estimate of the bioavailable fraction of metals; which will include both free metal ions and kinetically-
187 labile metal complexes (i.e., those with rapid dissociation kinetics) (Zhang and Davison, 1995). The
188 relationship between the concentrations of metals in blue mussels (both transplanted and native) and
189 labile metal concentrations measured with the DGT sampler is shown in Figure 3. Based on the
190 measurement of DGT-labile concentrations, blue mussel-water bioaccumulation factors for metals
191 measured in transplanted and native mussels could be calculated (Table 2). Interestingly, as shown in
192 Figure 4, the various metals detected in this study showed variable uptake patterns in transplanted and
193 native mussels, e.g. with concentrations of Hg (not analyzed in DGTs), Pb, Fe and Cd being relatively
194 higher in the native mussels than in the transplanted mussels whereas aluminum loads were generally
195 higher in the transplanted samples.

196 The bioaccumulation curves for the different PCB congeners detected in caged mussels at Station 1
197 varied significantly based on the degree of chlorination (and thus hydrophobicity) (Figure 5). As
198 shown in the results overview in Table 1, there was a noticeable difference in PCB concentration
199 levels between the pre-deployment mussel sample (which showed the lowest levels) and all other
200 mussel samples (both deployed and native), and the native mussels at Svensholmen displayed a

201 slightly higher PCB level than the deployed mussels at all time points (for PCB 7, $t = 6.45$, $p < 0.001$
202 in ANCOVA with time). Lipid-normalized concentrations of CB 138 and 101 showed a highly linear
203 increase over time for transplanted mussels at both sites (CB 101: $t = 3.11$, $p = 0.036$; CB 138: $t =$
204 5.04 , $p < 0.01$) (Figure 6). Some of the less chlorinated congeners (i.e. CB 28 and 31), however,
205 displayed a very different pattern, namely by increasing sharply during the first month before
206 decreasing during the following months. CB 101 and 118 showed an intermediate pattern by
207 apparently plateauing after 50 days of deployment at Svensholmen and after 100 days at the industrial
208 harbor (Figure 6). In the native mussels at Svensholmen, the PCB levels showed a slight but not
209 significant increase ($p > 0.2$) during the six months' study period, as shown in Table 1.

210 For PAHs, the highest concentrations were found in the native mussel samples (at Station 2,
211 Svensholmen), especially at the last sampling day towards the end of the study period (Table 1).
212 Unfortunately, at this last sampling point, the caging rig at the Svensholmen site had disappeared.
213 However, the key trend in native mussels was that all PAH concentrations decreased from May to
214 July, and then increased again to September and even more to November (Table 1, Figure 7). This was
215 most likely related to spawning and a resulting loss of tissue lipids in the early deployment period. For
216 PAHs in deployed mussels, there was a clear increase in concentration between pre-deployed to the
217 deployed groups (similar as for the PCBs), emphasizing the non-polluted nature of the donor
218 population at the Kaldvellfjord site. The two caging groups were slightly different, and interestingly,
219 Station 2 site exhibited higher concentrations than Station 1 ($t = 4.14$, $p < 0.01$; Table 1), i.e. an
220 opposite pattern to that seen for metals. The main uptake patterns of PAHs in caged mussels were
221 relatively similar at the two sites and concentrations of several PAHs (as pyrene, fluorene and
222 chrysene) increased linearly on lipid wt. basis at both sites (Figure 7). A comparison of the Station 2
223 native mussels to the pre-deployed mussels clearly show that the waters at Station 2 were quite
224 markedly contaminated with PAHs, with concentrations of some PAHs up to >50 times higher for
225 Station 2 native mussels (Table 1).

226 BAF values (wet wt.) for PAHs and PCBs in blue mussels are shown as a function of $\log K_{ow}$ in Figure
227 8, and the estimated BAF values for the different PCBs and PAHs are shown in Table 3 and Table 4,
228 respectively. In Figure 8, data are plotted against literature-based $\log K_{ow}$ - $\log BAF$ relationships from
229 Booij et al. (2006) and Smedes (2007). On average, absolute deviations between observed BAFs and
230 those from these empirical relationships from Booij et al and Smedes were on average 0.24 and 0.32
231 log units for PAHs and PCBs in native mussels of station 2. Average absolute deviations of observed
232 BAFs for transplanted mussels at station 1 and 2 were 0.40 and 0.35 log units and 0.32 and 0.41 log
233 units, respectively, when comparing with regressions curves from Booij et al. and Smedes.

234 Elimination rate constants for PAHs and PCBs in transplanted blue mussels at Svensholmen are shown
235 as a function of K_{ow} in Figure 9. The regression tool in Sigmaplot is used to obtain k_2 . The k_2 values
236 need to be treated with care as there is relatively large uncertainty in these values as shown by the

237 standard errors and P-values reported in Table 3 and Table 4. For PCBs, the k_2 values range between
238 0.016 d⁻¹ for CB 52 to 0.043 d⁻¹ for CB 101 in exposed blue mussels at Station 1 (Table 3); whereas for
239 the PAHs, the k_2 values range between 0.008 d⁻¹ for benzo[b,j]fluoranthene to 0.041 d⁻¹ for
240 fluoranthene in exposed blue mussels at Station 1 site (Table 4).

241 For Station 2, mussels of the final exposure period were lost, and therefore uptake curves for PAHs
242 and PCBs with logKow > 5 did not present significant plateauing, and modelling with Equation 1 was
243 difficult. Instead, we used an average of k_2 values from Station 1, C_m at $t = 0$ d and C_{Free} from passive
244 samplers to predict C_{mussel} at 34 and 104 d. The relationship between predicted/observed
245 concentrations in the deployed mussels at Station 2 and the hydrophobicity of the measured PAHs and
246 PCBs is shown in Figure 10. In general, deviations between observed and predicted PAH and PCB
247 concentrations in mussels are < 0.4 log unit, equivalent to no more than a factor of 2.5. Apart from the
248 least hydrophobic PAH (naphthalene) there appear to be an increasing predicted/observed ratio with
249 higher hydrophobicity.

250 4. Discussion

251 In the present study, non-contaminated blue mussels and passive sampler devices were deployed
252 alongside native mussels within a moderately polluted city harbor area (Kristiansand, Norway) both to
253 assess the contamination level at this location, compare the temporal pollutant bioaccumulation in
254 transplanted vs. native mussels, and also to estimate bioaccumulation parameters contaminants using a
255 first-order, single-compartment toxicokinetic approach.

256 At Station 1, the innermost harbor location, the finding of significantly increased nickel concentration
257 in caged mussels corroborates recent monitoring at this site (Schøyen and Håvardstun, 2016) and is
258 also supported by the fact that the site is in the vicinity of a nickel processing plant. According to data
259 from caged mussels, Station 1 was generally more contaminated than Station 2, except for PAHs.
260 Interestingly, Station 2 was located relatively close to the Fiskå bay area (Figure 1) which is home to
261 an industrial company that earlier was known to be the main source of PAH pollution to the
262 Kristiansand harbor waters. The finding was also strengthened by analyses of native mussels at Station
263 2, which showed elevated PAH levels as compared with typical background levels and with levels
264 measured in the 0-group mussel. For metals, the analysis results in caged mussels indicated that a
265 putative steady state was reached relatively fast, and generally faster than the non-polar, hydrophobic
266 organic contaminants. A one-month deployment seemed to be long enough for stable concentration to
267 be established. For the hydrophobic organic contaminants (PCBs and PAHs), a linear bioaccumulation
268 occurred during the first months of caging, but the deployed mussels did in general not reach the
269 concentrations detected in the native mussels, not even after six months of deployment. However,
270 some of the least hydrophobic PCBs and PAHs (e.g. CB 28 and naphthalene) showed a different

271 pattern with a plateauing tendency already after one month, when contamination data on wet wt. basis
272 were used, indicating the reaching of a steady state for these congeners. But this plateauing tendency
273 was not seen for lipid-normalized concentrations.

274 The native mussel population at the Station 2 site was analyzed repeatedly during the six months'
275 study period and these data clearly indicated the confounding influence from seasonal fluctuations on
276 biological parameters (lipid %) as well as on chemical contaminant endpoints (especially PAHs but
277 also some of the PCBs). In retrospect, repeated sampling and analyses of mussels from the donor-
278 population (the Kaldvellfjord site) at each sampling date during the whole six months' study period
279 would have provided a better basis for assessing the confounding influence from seasonal fluctuations
280 in the present study. The PAH level in the native mussels decreased in the early phase of the study to a
281 minimum during summer and then increased again towards the last sampling point in November.
282 Many studies emphasize the relevance of considering seasonal fluctuations when interpreting
283 contaminant data (and also biomarker signals) in blue mussels, e.g. (Björk and Gilek, 1997;
284 Westerbom et al., 2002; Orban et al., 2002; Pfeifer et al., 2005; Leinio and Lehtonen, 2005; Nesto et
285 al., 2007; Farcy et al., 2013; Schmidt et al., 2013; Mugica et al., 2015). The annual cycle will in a
286 complex and dynamic manner influence pollutant bioaccumulation processes that occur *in situ* and the
287 biological condition and pollution responses that appear *in vivo* in mussels. In this regard, variability
288 in nutritional/growth and reproductive (e.g. spawning and gametogenesis) factors are important,
289 regardless of whether the endpoints of the study are chemical exposure markers or ecotoxicological
290 effect markers. Nevertheless, the mussel sentinels deployed in the Kristiansand harbor in the present
291 study, rapidly changed from the pre-deployment level by accumulating increased levels of
292 ecotoxicologically relevant metals, PAHs and PCBs, although most of the PAHs showed consistently
293 lower concentrations than the levels found in the native mussel collected from the Svensholmen site.
294 However, it may be argued that steady-state conditions may not need to be reached for deployed
295 mussels if the objectives of the study are to compare contamination levels at different field sites or for
296 establishing time trends, so long as the kinetics of accumulation are the same at all sites (i.e. same time
297 of year, similar water temperature, same deployment design, etc.). The generally higher PAH levels in
298 native mussels than in transplanted mussels in the present study is most likely because the native
299 mussels had a much longer time of exposure (several years). The long-term bioaccumulation of PAHs
300 in native mussels is a complex process and the modelling of this process requires the use of multi-
301 compartment uptake and elimination modelling tools, e.g. (Stegeman and Teal, 1973), or toxicokinetic
302 models such as those based on dynamic energy budget (DEB) theory, e.g. (Vanharen and Kooijman,
303 1993; Vanharen et al., 1994), which can estimate the dynamic influence on bioaccumulation by
304 multiple factors related both to the pollutant, the environment, and the physiological condition of the
305 sentinel organism.

306 However, first-order, single-compartment models are still the most common tools in the
307 ecotoxicological studies for studies of contaminant bioaccumulation and for estimating BAFs. So,
308 what could be the best endpoint for indicating that steady-state contaminant concentrations are
309 obtained in deployed mussels: an observed plateauing of C_m/C_{free} or the determination of identical C_m
310 in native and transplanted mussels? In our study, there was apparently a systematic trend towards a
311 higher predicted/observed ratio with higher K_{ow} values within both the PCBs and PAHs chemical
312 classes (Figure 10). Other studies have found that the linear relationship between $\log BAF$ and $\log K_{ow}$
313 does not hold for compounds with $\log K_{ow} > 6$. Devillers et al. (1996), and Barthe et al. (2008)
314 suggested that this phenomenon is caused by steric hindrance of permeation through biological
315 membranes by the larger (and higher K_{ow}) contaminant molecules. Here, the accumulation and
316 depuration rates were different for PAHs and PCBs. The slope of the regression of $\log BAF$ against
317 $\log K_{ow}$ was slightly lower than 1 (0.92 for PCBs and 0.76 for PAHs) (Table 5), which is similar to that
318 reported by Booij et al. (2006) in a review study that addressed the three mussel species *Mytilus*
319 *edulis*, *M. complanata*, and *Perna viridis*. The intercepts in our study are apparently a bit lower than
320 those reported by Booij et al. (2006) and it should be noted that the reported ratios span 4 orders of
321 magnitude. The BAF values obtained in this study are consistent with the values by the Booij et al.
322 (2006) review. As expected from contaminant masses found in native and transplanted mussels,
323 differences in BAFs can be seen for native and transplanted mussels.

324 The time required for deployed mussels to reach steady state for different contaminants will obviously
325 depend on the toxicokinetic properties of the specific pollutant substance, on factors attributed to the
326 mussel (condition, reproductive state, etc.), as well as on recipient factors *in situ*. While the uptake of
327 hydrophobic (non-polar) organic contaminants, such as PCBs and PAHs, occur as a passive diffusive
328 process/equilibrium partitioning, other and more complex mechanisms are thought to be involved for
329 trace metals, as their accumulation by mussel and other aquatic organisms is influenced by a variety of
330 factors, such as multiple routes of exposure (diet and solution), metal speciation, ligand associations
331 and complexation, chemical composition of the surrounding medium and physiological or biochemical
332 effects on bioavailability (Luoma, 1983; Simkiss and Taylor, 1989; Luoma and Rainbow, 2005).
333 According to Jenne (1977), the bioavailability of trace metals to mussels may be influenced by at least
334 four factors: (1) the physiological and ecological characteristics of the mussels, (2) the forms of
335 dissolved elements, (3) the forms of elements in ingested solids, and (4) the chemical and
336 physiological characteristics of the seawater. For instance, Cd entry into cells of gills of marine
337 mollusks may occur through calcium channels (Roesijadi and Unger, 1993). Some elements are also
338 essential, meaning that they are necessary for optimal growth, development and homeostasis. As such,
339 organisms are capable of regulating these metals (Lobel and Marshall, 1988). Differences in
340 accumulation between transplanted and native mussels may therefore be a consequence of
341 physiological differences (e.g. pertaining to their general condition), resulting in different uptake

342 and/or depuration rates for specific elements. This could be related e.g. to different ingestion rates,
343 different transport through ion channels at the cell membrane, or different concentrations of metal-
344 binding proteins, such as metallothionein. Cd may e.g. compete with Ca for transmembrane transport
345 by calcium pump in the epithelia of mussels. The rapid bioaccumulation of trace metals in caged
346 mussels in the present study, in particular at Station 1, has also been observed by others. Regoli and
347 Orlando (1994) studied the uptake of Pb, Fe, and Mn in *Mytilus galloprovincialis* deployed at a metal
348 polluted site and reported that a steady state was reached after only 2 weeks, suggesting that mussel
349 rapidly equilibrate with the elevated environmental levels of metal pollutants. It can be expected that
350 native and transplanted mussels experience exposure to the same forms/species of the elements
351 (dissolved or associated with solids) and water characteristics, however, these may change with time,
352 and thus explain some of the differences observed between mussels sampled in July (34 days) and
353 mussels sampled in September (104 days). With respect to PCBs and PAHs, there was a good
354 correspondence in the present study between levels determined in mussels and levels determined in
355 water through the use of passive samplers. Moreover, the observed bioaccumulation in mussels
356 revealed only little systematic difference between PCBs and PAHs (e.g. Figure 8, Figure 9, and Figure
357 10). The correspondence between mussels and passive samplers for PCBs and PAHs was actually
358 better than expected, especially for the PAHs which in these industrially influenced harbor waters can
359 be expected to be predominantly associated with microscopic coal tar pitch and soot particles of a
360 local industrial origin. Such particles may contain PAHs both adsorbed to the particle surface as well
361 as absorbed in the internal particle matrix, and they are key factors for the partitioning, bioavailability,
362 uptake, and bioaccumulation of PAHs in aquatic environments (Gustafsson and Gschwend, 1997;
363 Gustafsson et al., 1997; Cornelissen et al., 2005). In the present study, PAHs from a predominately
364 soot particle origin would be expected to be less available for uptake by passive samplers in
365 comparison to by the mussels, as the active filter feeding process of mussels will provide more routes
366 for uptake (both from diet and solution). Importantly, as the mussels in this study were not depurated
367 prior to analysis, PAH associated to particles contained in the mussel gut must have contributed to the
368 detected concentration level. However, the observed high degree of correspondence between PCB and
369 PAH accumulation in the mussels supports an assumption that passive uptake from the solved fraction
370 was the dominating uptake route for both contaminant classes. And this uptake route is also what is
371 measured by the passive samplers.

372 In summary, a general increase of contaminant loads was recorded when clean blue mussels were
373 deployed in the waters of the Kristiansand harbor. The increase was most significant for certain metals
374 (Ni and Cu) at Station 1 (the industrial site), which is in agreement with our previous monitoring data
375 of this site. At Station 2, the measured PAH levels (in both caged and native mussels) were markedly
376 higher than at the Station 1, which most likely was due to the closer proximity of Station 2 site to
377 another industrialized site which historically was (and probably still is) the main source for PAH

378 contamination to the Kristiansand harbor waters. The native mussels present at Station 2 were
379 significantly more contaminated by PAHs and PCBs than the mussels deployed in the harbor study
380 area, also after six months' deployment. The high K_{ow} PAHs displayed a higher native - transplant
381 ratio than the trace metals, indicating that a longer time than six months is required for steady state to
382 establish. In order to establish steady state for all monitoring-relevant contaminants, the caged mussels
383 will probably need to be deployed for exposure times that are in the range of the age of native mussels.
384 Although this is possible to achieve, it will not be practically feasible for most mussel monitoring
385 studies. Rather, from a practical viewpoint, short mussel deployments are by most means desirable as
386 it reduces both field costs as well as the risk of practical problems such as biofouling or the loss of
387 caging rigs. Our results suggest that the optimal deployment time in mussel caging is highly substance
388 dependent, with the most hydrophobic organic contaminants requiring very long deployments. Short
389 term mussel deployments, such as one-two months, appear to be suitable for trace metals and the less
390 hydrophobic non-polar organic contaminants. However, such quite short deployments may also work
391 fine for monitoring of other, more hydrophobic, organic contaminants as long as the study aim is to
392 compare time trends and relative exposure loads at different field sites, and not to describe
393 contaminant concentrations under real steady state conditions. For the latter issue, the use of very long
394 mussel deployments would be recommended, if the collection and analysis of native mussel sentinels
395 is not possible at the study site. Otherwise, a toxicokinetic modelling approach must be used to predict
396 theoretical steady state levels from contaminant concentration data in blue mussels that have been
397 deployed only for short periods of time or from passive samplers, or both.

398 **Acknowledgments**

399 This study is funded by the Norwegian Research Council through NIVA's institutional funding and
400 the Norwegian Environmental Agency via the National Function programme. The authors want to
401 acknowledge John Arthur Berge, Lise Tveiten and Alfhild Kringstad for important contributions
402 during planning, fieldwork and analysis, respectively. The authors also acknowledge the reviewers of
403 this journal for their valuable recommendations.

404 **References:**

- 405 Allan, I.J., Harman, C., Ranneklev, S.B., Thomas, K.V., Grung, M., 2013. Passive sampling for target and
406 nontarget analyses of moderately polar and nonpolar substances in water. *Environmental Toxicology*
407 *and Chemistry* 32, 1718-1726.
- 408 Barthe, M., Pelletier, E., Breedveld, G.D., Cornelissen, G., 2008. Passive samplers versus surfactant
409 extraction for the evaluation of PAH availability in sediments with variable levels of contamination.
410 *Chemosphere* 71, 1486-1493.

- 411 Bebianno, M.J., Lopes, B., Guerra, L., Hoarau, P., Ferreira, A.M., 2007. Glutathione S-transferases and
412 cytochrome P450 activities in *Mytilus galloprovincialis* from the South coast of Portugal: Effect of abiotic
413 factors. *Environ. Int.* 33, 550-558.
- 414 Beyer, J., Green, N., Brooks, S., Allan, I., Ruus, A., Gomes, T., Brate, I., Schøyen, M., this volume. Blue
415 mussels (*Mytilus edulis* spp.) as sentinel organisms in coastal pollution monitoring: A review. *Marine*
416 *Environmental Research*.
- 417 Bignert, A., Eriksson, U., Nyberg, E., Miller, A., Danielsson, S., 2014. Consequences of using pooled
418 versus individual samples for designing environmental monitoring sampling strategies. *Chemosphere*
419 94, 177-182.
- 420 Björk, M., Gilek, M., 1997. Bioaccumulation kinetics of PCB 31, 49 and 153 in the blue mussel, *Mytilus*
421 *edulis* L as a function of algal food concentration. *Aquatic Toxicology* 38, 101-123.
- 422 Björk, M., Gilek, M., 1999. Efficiencies of polychlorinated biphenyl assimilation from water and algal food
423 by the blue mussel (*Mytilus edulis*). *Environmental Toxicology and Chemistry* 18, 765-771.
- 424 Bodin, N., Burgeot, T., Stanisiere, J.Y., Bocquene, G., Menard, D., Minier, C., Boutet, I., Amat, A., Chereil,
425 Y., Budzinski, H., 2004. Seasonal variations of a battery of biomarkers and physiological indices for the
426 mussel *Mytilus galloprovincialis* transplanted into the northwest Mediterranean Sea. *Comparative*
427 *Biochemistry and Physiology C-Toxicology & Pharmacology* 138, 411-427.
- 428 Booij, K., Smedes, F., van Weerlee, E.M., Honkoop, P.J.C., 2006. Environmental monitoring of
429 hydrophobic organic contaminants: The case of mussels versus semipermeable membrane devices.
430 *Environmental Science & Technology* 40, 3893-3900.
- 431 Borja, A., Elliott, M., Carstensen, J., Heiskanen, A.S., van de Bund, W., 2010. Marine management -
432 Towards an integrated implementation of the European Marine Strategy Framework and the Water
433 Framework Directives. *Marine Pollution Bulletin* 60, 2175-2186.
- 434 Brooks, S., Harman, C., Soto, M., Cancio, I., Glette, T., Marigomez, I., 2012. Integrated coastal
435 monitoring of a gas processing plant using native and caged mussels. *Science of the Total*
436 *Environment* 426, 375-386.
- 437 Cornelissen, G., Gustafsson, O., Bucheli, T.D., Jonker, M.T.O., Koelmans, A.A., Van Noort, P.C.M.,
438 2005. Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and
439 soils: Mechanisms and consequences for distribution, bioaccumulation, and biodegradation.
440 *Environmental Science & Technology* 39, 6881-6895.
- 441 Dabrin, A., Ghestem, J.P., Uher, E., Gonzalez, J.L., Allan, I.J., Schintu, M., Montero, N., Balaam, J.,
442 Peinerud, E., Miega, C., Coquery, M., 2016. Metal measurement in aquatic environments by passive
443 sampling methods: Lessons learning from an *in situ* intercomparison exercise. *Environmental Pollution*
444 208, 299-308.
- 445 Devillers, J., Bintein, S., Domine, D., 1996. Comparison of BCF models based on log P. *Chemosphere* 33,
446 1047-1065.
- 447 Ericson, G., Skarphéinsdóttir, H., Dalla Zuanna, L., Svavarsson, J., 2002. DNA adducts as indicators of
448 genotoxic exposure in indigenous and transplanted mussels, *Mytilus edulis* L. from Icelandic coastal
449 sites. *Mutation Research-Genetic Toxicology and Environmental Mutagenesis* 516, 91-99.
- 450 Farcy, E., Burgeot, T., Haberkorn, H., Auffret, M., Lagadic, L., Allenou, J.-P., Budzinski, H., Mazzella, N.,
451 Pete, R., Heydorff, M., Menard, D., Mondeguer, F., Caquet, T., 2013. An integrated environmental
452 approach to investigate biomarker fluctuations in the blue mussel *Mytilus edulis* L. in the Vilaine estuary,
453 France. *Environmental Science and Pollution Research* 20, 630-650.
- 454 Farrington, J.W., Tripp, B.W., Tanabe, S., Subramanian, A., Sericano, J.L., Wade, T.L., Knap, A.H., 2016.
455 Edward D. Goldberg's proposal of "the Mussel Watch": Reflections after 40 years. *Marine Pollution*
456 *Bulletin* 110, 501-510.
- 457 Green, N., Dye, C., Remberger, M., Schlabach, M., Herzke, D., Schøyen, M., Bakke, T., Huber, S.,
458 Uggerud, H., Brevik, E., Plosz, B., Vogelsang, C., 2008. Screening of selected metals and new organic
459 contaminants 2007. Norwegian Pollution Control Authority, Oslo Norway, p. 104.

- 460 Green, N.W., Schøyen, M., Øxnevad, S., Ruus, A., Allan, I., Hjermann, D., Severinsen, G., Høgåsen, T.,
461 Beylich, B., Håvardstun, J., Lund, E., Tveiten, L., Bæk, K., 2016. Contaminants in coastal waters of
462 Norway - 2015. Norwegian Environment Agency Miljødirektoratet & Norwegian Institute for Water
463 Research, Oslo, Norway, p. 209 pp.
- 464 Gustafsson, O., Gschwend, P.M., 1997. Soot as a strong partition medium for polycyclic aromatic
465 hydrocarbons in aquatic systems, in: Eganhouse, R.P. (Ed.), *Molecular Markers in Environmental*
466 *Geochemistry*. Amer Chemical Soc, Washington, pp. 365-381.
- 467 Gustafsson, O., Haghseta, F., Chan, C., MacFarlane, J., Gschwend, P.M., 1997. Quantification of the
468 dilute sedimentary soot phase: Implications for PAH speciation and bioavailability. *Environmental*
469 *Science & Technology* 31, 203-209.
- 470 Jenne, E.A., Luoma, S.N., 1977. Forms of trace elements in soil, sediments and waters: an overview of
471 their determination and biological availability., in: Wildung, R.E., Druckers, H. (Eds.), *Biological*
472 *implications of metals in the environment*. National Technical Information Service, Springfield, pp.
473 110-143.
- 474 Leinio, S., Lehtonen, K.K., 2005. Seasonal variability in biomarkers in the bivalves *Mytilus edulis* and
475 *Macoma balthica* from the northern Baltic Sea. *Comparative Biochemistry and Physiology C-Toxicology*
476 *& Pharmacology* 140, 408-421.
- 477 Lobel, P.B., Marshall, H.D., 1988. A unique low-molecular weight zinc-binding ligand in the kidney
478 cytosol of the mussel *Mytilus-edulis*, and its relationship to the inherent variability of zinc accumulation
479 in this organism. *Marine Biology* 99, 101-105.
- 480 Luoma, S.N., 1983. Bioavailability of trace-metals to aquatic organisms - a review. *Science of the Total*
481 *Environment* 28, 1-22.
- 482 Luoma, S.N., Rainbow, P.S., 2005. Why is metal bioaccumulation so variable? Biodynamics as a unifying
483 concept. *Environmental Science & Technology* 39, 1921-1931.
- 484 Mugica, M., Sokolova, I.M., Izagirre, U., Marigomez, I., 2015. Season-dependent effects of elevated
485 temperature on stress biomarkers, energy metabolism and gamete development in mussels. *Marine*
486 *Environmental Research* 103, 1-10.
- 487 Nasci, C., Nesto, N., Monteduro, R.A., Da Ros, L., 2002. Field application of biochemical markers and a
488 physiological index in the mussel, *Mytilus galloprovincialis*: transplantation and biomonitoring studies in
489 the lagoon of Venice (NE Italy). *Marine Environmental Research* 54, 811-816.
- 490 Nesto, N., Romano, S., Moschino, V., Mauri, M., Da Ros, L., 2007. Bioaccumulation and biomarker
491 responses of trace metals and micro-organic pollutants in mussels and fish from the Lagoon of Venice,
492 Italy. *Marine Pollution Bulletin* 55, 469-484.
- 493 Nigro, M., Falleni, A., Del Barga, I., Scarcelli, V., Lucchesi, P., Regoli, F., Frenzilli, G., 2006. Cellular
494 biomarkers for monitoring estuarine environments: Transplanted versus native mussels. *Aquatic*
495 *Toxicology* 77, 339-347.
- 496 Orban, E., Di Lena, G., Nevigato, T., Casini, I., Marzetti, A., Caproni, R., 2002. Seasonal changes in meat
497 content, condition index and chemical composition of mussels (*Mytilus galloprovincialis*) cultured in two
498 different Italian sites. *Food Chemistry* 77, 57-65.
- 499 OSPARCOM, 2012. JAMP Guidelines for Monitoring Contaminants in Biota. OSPAR Commission,
500 London, p. 122 pp.
- 501 Peven, C.S., Uhler, A.D., Querzoli, F.J., 1996. Caged mussels and semipermeable membrane devices as
502 indicators of organic contaminant uptake in Dorchester and Duxbury Bays, Massachusetts.
503 *Environmental Toxicology and Chemistry* 15, 144-149.
- 504 Pfeifer, S., Schiedek, D., Dippner, J.W., 2005. Effect of temperature and salinity on acetylcholinesterase
505 activity, a common pollution biomarker, in *Mytilus sp* from the south-western Baltic Sea. *Journal of*
506 *Experimental Marine Biology and Ecology* 320, 93-103.

- 507 Regoli, F., Orlando, E., 1994. Accumulation and subcellular-distribution of metals (Cu, Fe, Mn, Pb and
508 Zn) in the Mediterranean mussel *Mytilus-galloprovincialis* during a field transplant experiment. Marine
509 Pollution Bulletin 28, 592-600.
- 510 Regoli, F., Principato, G., 1995. Glutathione, glutathione-dependent and antioxidant enzymes in mussel,
511 *Mytilus-galloprovincialis*, exposed to metals under field and laboratory conditions - implications for the
512 use of biochemical biomarkers. Aquatic Toxicology 31, 143-164.
- 513 Roesijadi, G., Unger, M.E., 1993. Cadmium uptake in gills of the mollusk *Crassostrea-virginica* and inhibition
514 by calcium-channel blockers. Aquatic Toxicology 24, 195-206.
- 515 Schmidt, W., Power, E., Quinn, B., 2013. Seasonal variations of biomarker responses in the marine blue
516 mussel (*Mytilus spp.*). Marine Pollution Bulletin 74, 50-55.
- 517 Schøyen, M., Håvardstun, J., 2016. Operational monitoring in compliance with the EU Water Framework
518 Directive for Glencore Nikkelverk AS in Kristiansandsfjord in 2014/2015. Investigations of blue
519 mussel and sediments. (Report in Norwegian with English summary). Norwegian Institute for Water
520 Research (NIVA), Oslo, Norway, p. 82 pp.
- 521 Serafim, A., Lopes, B., Company, R., Cravo, A., Gomes, T., Sousa, V., Bebianno, M.J., 2011. A multi-
522 biomarker approach in cross-transplanted mussels *Mytilus galloprovincialis*. Ecotoxicology 20, 1959-1974.
- 523 Simkiss, K., Taylor, M.G., 1989. Metal fluxes across membranes of aquatic organisms. Reviews in Aquatic
524 Sciences 1, 173-188.
- 525 Smedes, F., 2007. Monitoring of chlorinated biphenyls and polycyclic aromatic hydrocarbons by passive
526 sampling in concert with deployed mussels, in: Greenwood, R., Mills, G., Vrana, B. (Eds.), Passive
527 Sampling Techniques in Environmental Monitoring. Elsevier, Amsterdam, pp. 407-448.
- 528 Stegeman, J.J., Teal, J.M., 1973. Accumulation, release and retention of petroleum hydrocarbons by the
529 oyster *Crassostrea virginica*. Marine Biology 22, 37 - 44.
- 530 Vanharen, R.J.F., Kooijman, S.A.L.M., 1993. Application of a dynamic energy budget model to *Mytilus-*
531 *edulis* (L). Neth. J. Sea Res. 31, 119-133.
- 532 Vanharen, R.J.F., Schepers, H.E., Kooijman, S., 1994. Dynamic energy budgets affect kinetics of
533 xenobiotics in the marine mussel *Mytilus-edulis*. Chemosphere 29, 163-189.
- 534 Walsh, A.R., O'Halloran, J., 1998. Accumulation of chromium by a population of mussels (*Mytilus edulis*
535 (L.)) exposed to leather tannery effluent. Environmental Toxicology and Chemistry 17, 1429-1438.
- 536 Westerborn, M., Kilpi, M., Mustonen, O., 2002. Blue mussels, *Mytilus edulis* at the edge of the range:
537 population structure, growth and biomass along a salinity gradient in the north-eastern Baltic Sea.
538 Marine Biology 140, 991-999.
- 539 Zhang, H., Davison, W., 1995. Performance-characteristics of diffusion gradients in thin-films for the *in-*
540 *situ* measurement of trace-metals in aqueous-solution. Anal. Chem. 67, 3391-3400.
- 541
- 542

544 Legends of figures:

545 **Figure 1. Localization of the two mussel stations 1 and 2 (industrial site and Svensholmen) in the harbor area of the**
546 **Kristiansand city, southern Norway.**

547 **Figure 2. Temporal changes in trace element concentrations in transplanted blue mussels (mg kg^{-1} wet wt.) exposed at**
548 **Station 1 (industrial site) (○) and at Station 2 (Svensholmen) (□) for deployment times of 34, 104 and 181 days. Note**
549 **the different scales on the y-axes.**

550 **Figure 3. Concentrations of trace elements (mg kg^{-1} wet wt.) in transplanted blue mussels at Station 1 (industrial site)**
551 **and Station 2 (Svensholmen) (after 34, 104 and 181 days of exposure) and in native blue mussels (Station 2 only;**
552 **sampled on 4 occasions) as a function of DGT-labile concentrations ($\mu\text{g L}^{-1}$).**

553 **Figure 4. Ratio of trace element concentrations in transplanted blue mussels over those in native blue mussels at**
554 **Station 2 sampled on July 2nd (●) and Sept. 10th 2015 (○). Error bars are calculated from relative percent deviations**
555 **of duplicate analyses for transplanted and native mussels.**

556 **Figure 5. PCB accumulation curves in transplanted blue mussels exposed at Station 1 (industrial site). The y-axis**
557 **C_m/C_{free} (L kg^{-1}) represents the concentration in mussels (C_m in ng g^{-1} wet wt.) over the freely dissolved concentration**
558 **in water (ng L^{-1}) determined by passive sampling for each sampling time (0, 34, 104 and 181 days). Duplicate pooled**
559 **mussel samples were analyzed. See material and methods section (section 2.3) and Equation 1 for model lines.**

560 **Figure 6. Accumulation curves over time for PCBs on wet (w.) and lipid (l.) wt. basis in transplanted mussels at**
561 **Station 1 (industrial site), and both transplanted and native mussels at Station 2 (Svensholmen). Curve fitting was for**
562 **visual impression and has no mathematical meaning.**

563 **Figure 7. Accumulation curves over time for PAHs on wet (w.) and lipid (l.) wt. basis in transplanted mussels at**
564 **Station 1 (industrial site), and both transplanted and native mussels at Station 2 (Svensholmen). Curve fitting was for**
565 **visual impression and has no mathematical meaning.**

566 **Figure 8. Logarithm of bioaccumulation factor (log BAF, calculated as the contaminant concentration wet wt. in blue**
567 **mussels over the freely dissolved concentration) for PAHs (empty symbols) and PCBs (filled symbols) for native**
568 **mussels at Station 2 (Svensholmen), transplanted mussels at Station 2 and transplanted mussels at Station 1**
569 **(industrial site) as a function of $\log K_{ow}$. See the text for derivation of log BAF values. Error bars for log BAF for**
570 **native mussels from Station 2 represent standard deviation calculated from log BAF estimated at time 0, 34, 104, and**
571 **181 days of the experiment ($n = 4$). The solid and dashed lines represent logBAF-logKow regressions from Booij et al.**
572 **(2006) ($\log \text{BAF} = 0.84 \log K_{ow} - 0.49$) and Smedes (2007) ($\log \text{BAF} = 1.1 \log K_{ow} - 2.14$), respectively.**

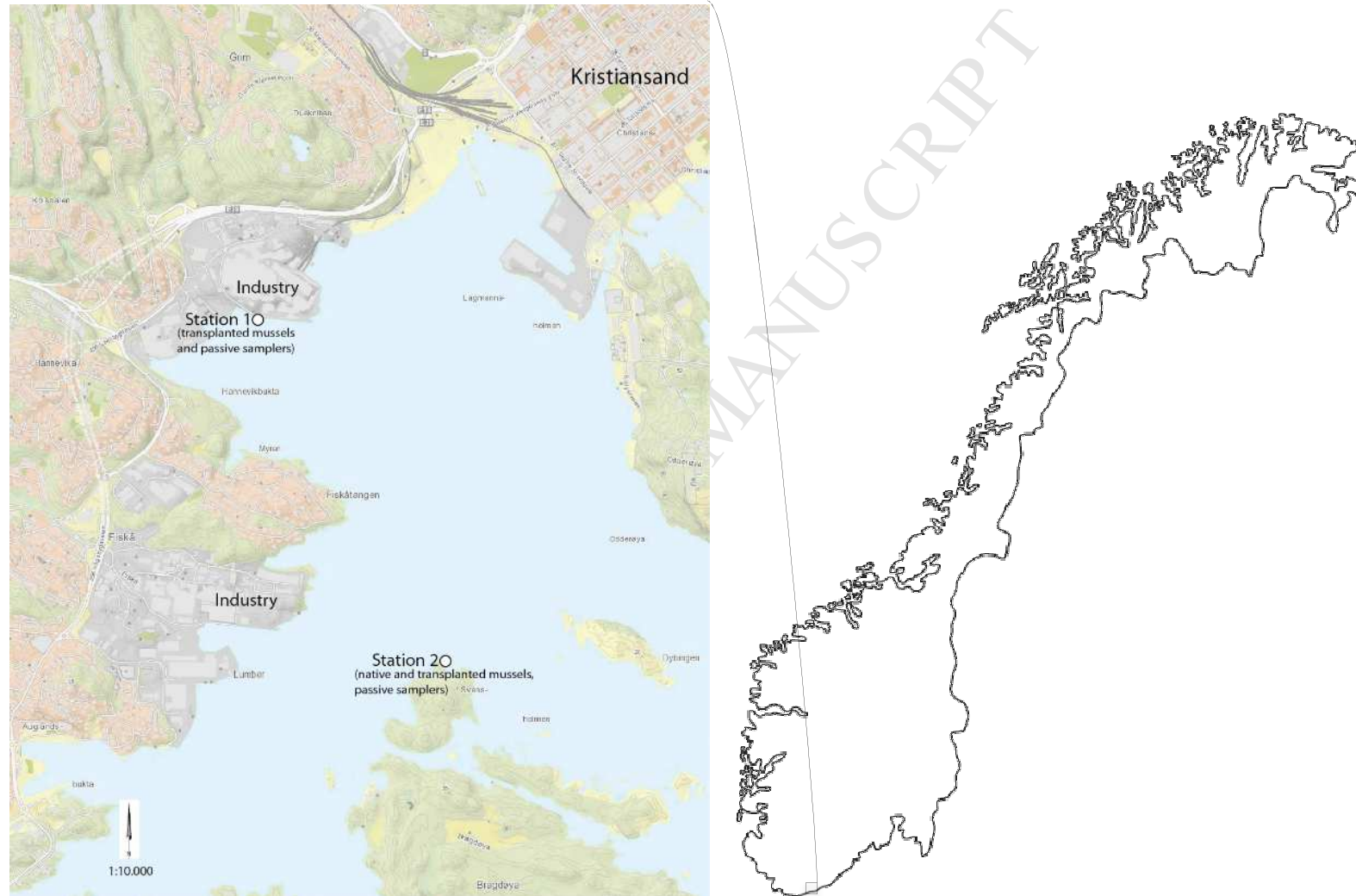
573 **Figure 9. First-order depuration rate constants, k_2 for PAHs and PCBs in transplanted blue mussels exposed at**
574 **Station 2 (Svensholmen). The regression shown is from Booij et al. (2006).**

575 **Figure 10. Logarithm of the ratio of predicted PCB and PAH concentrations as a function of K_{OW} in blue mussels over**
576 **observed concentrations for transplanted blue mussel exposures of 34 and 104 days at Station 2 (Svensholmen).**

577

578

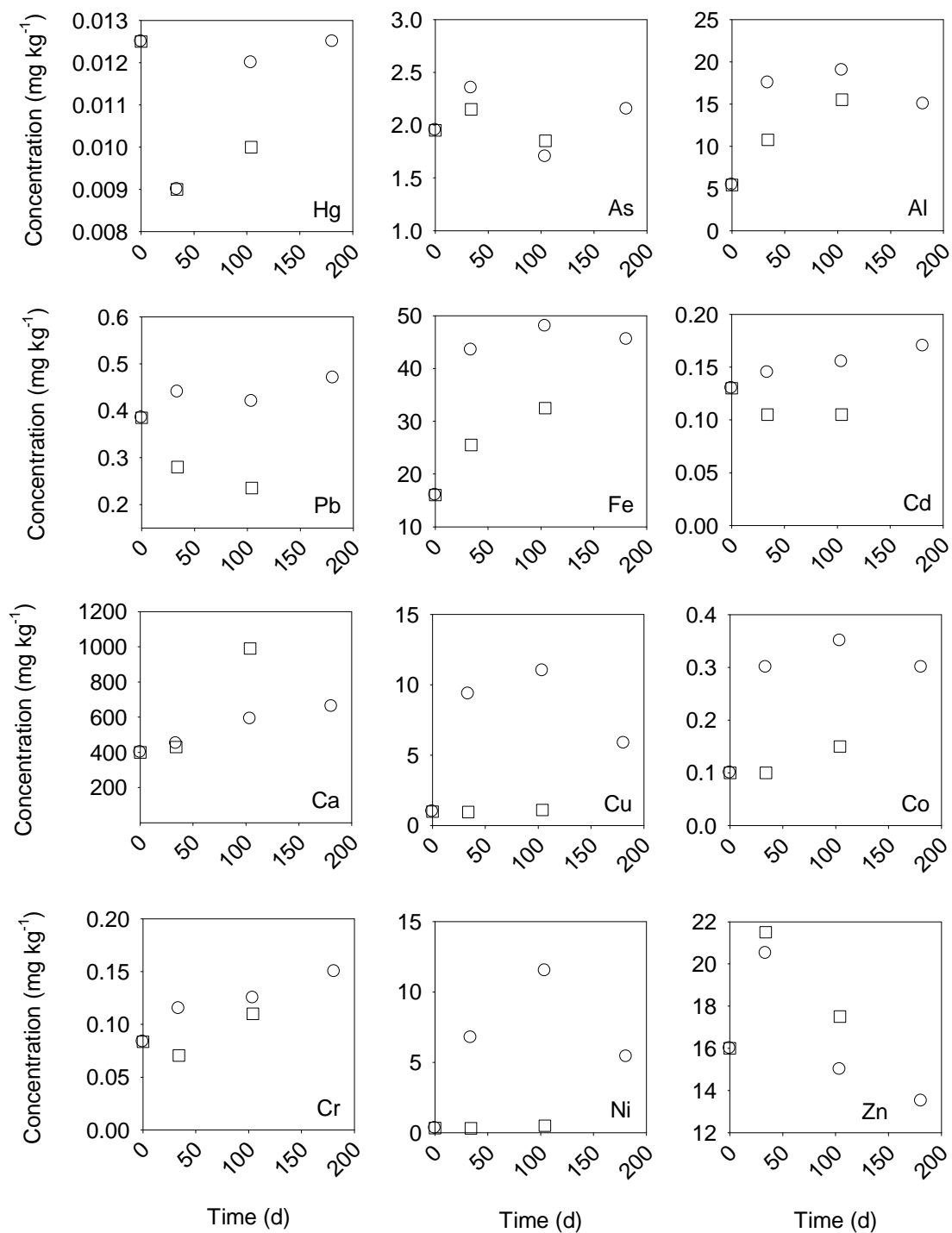
579 Figure 1



580

581

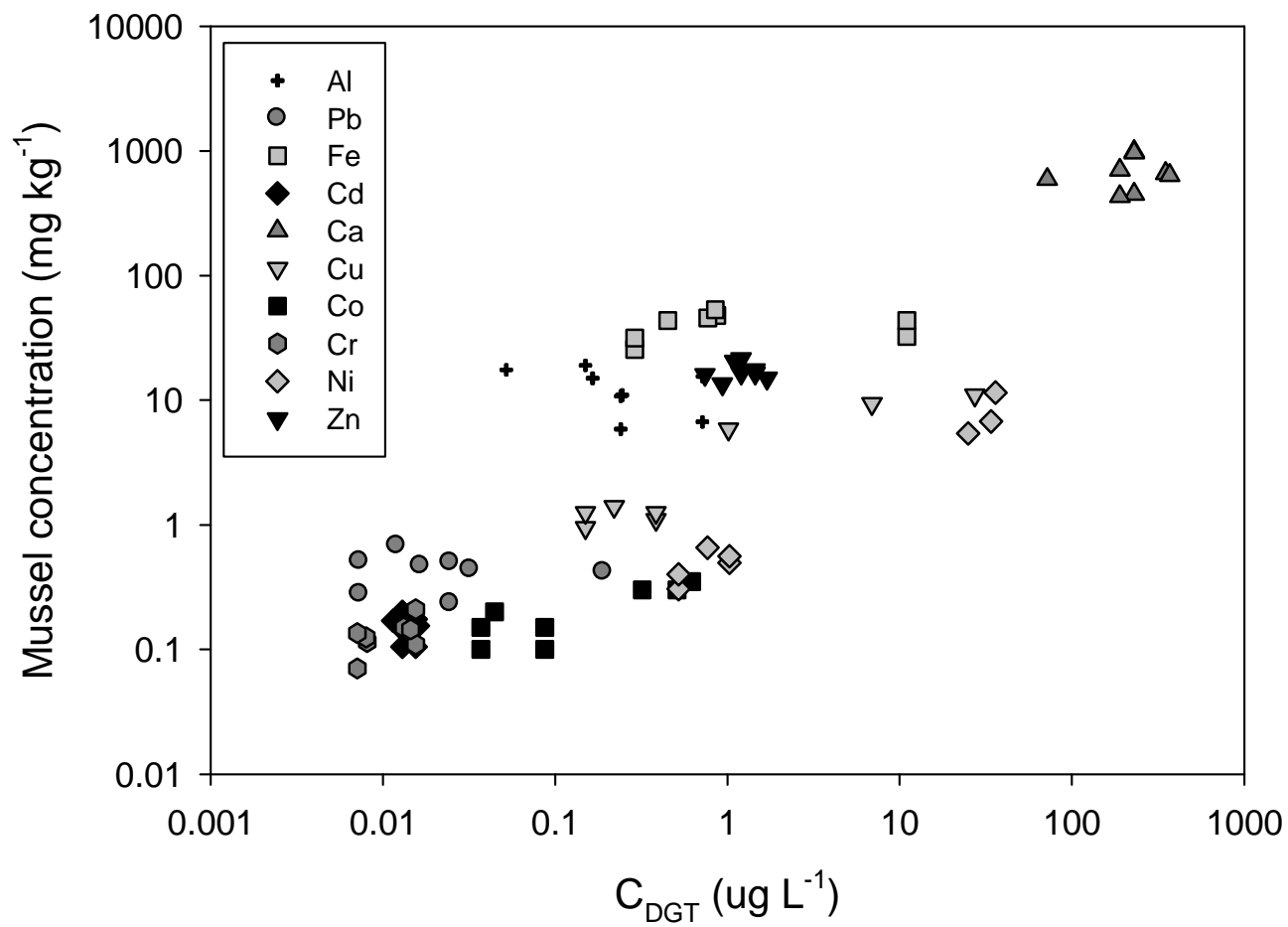
582 Figure 2



583

584

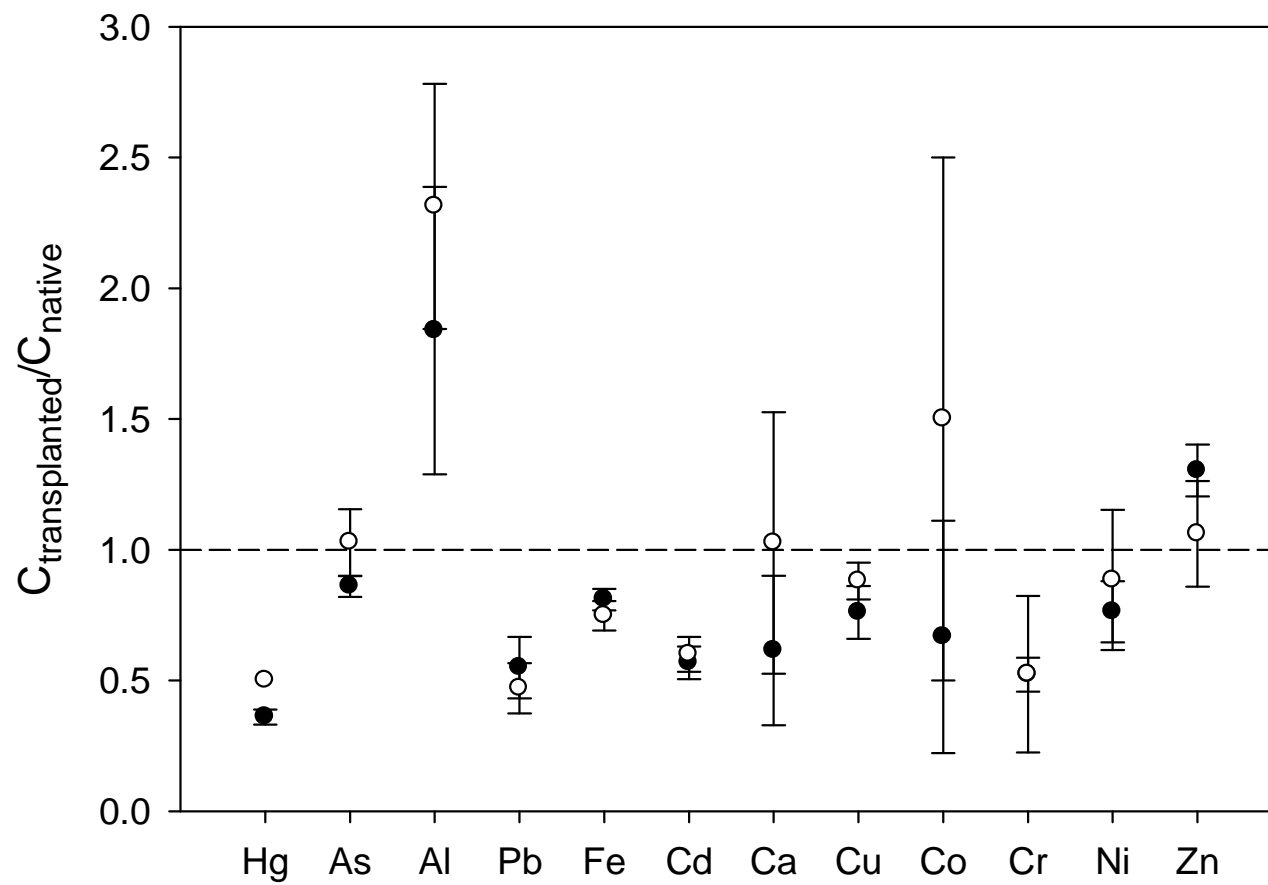
585 Figure 3



586

587

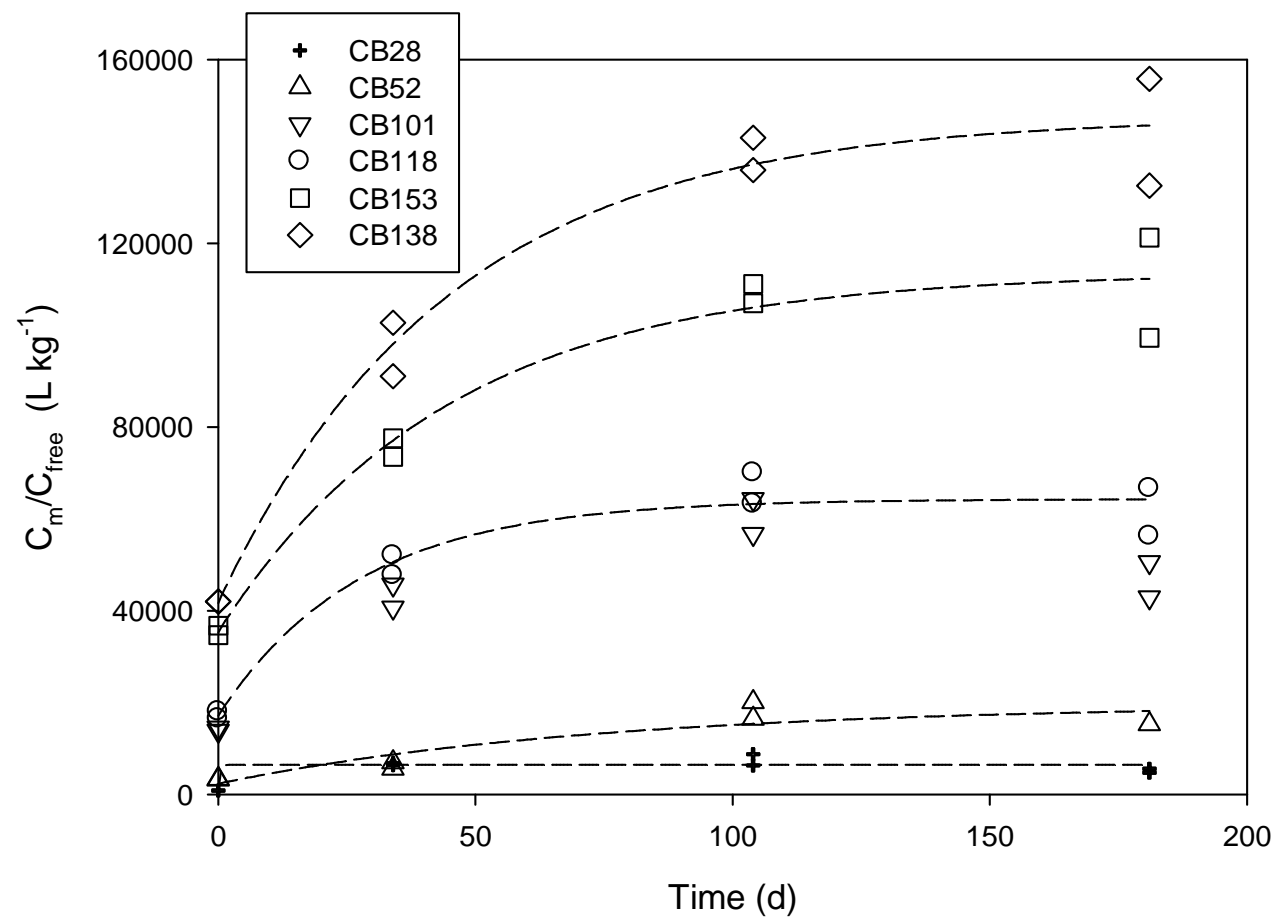
588 Figure 4



589

590

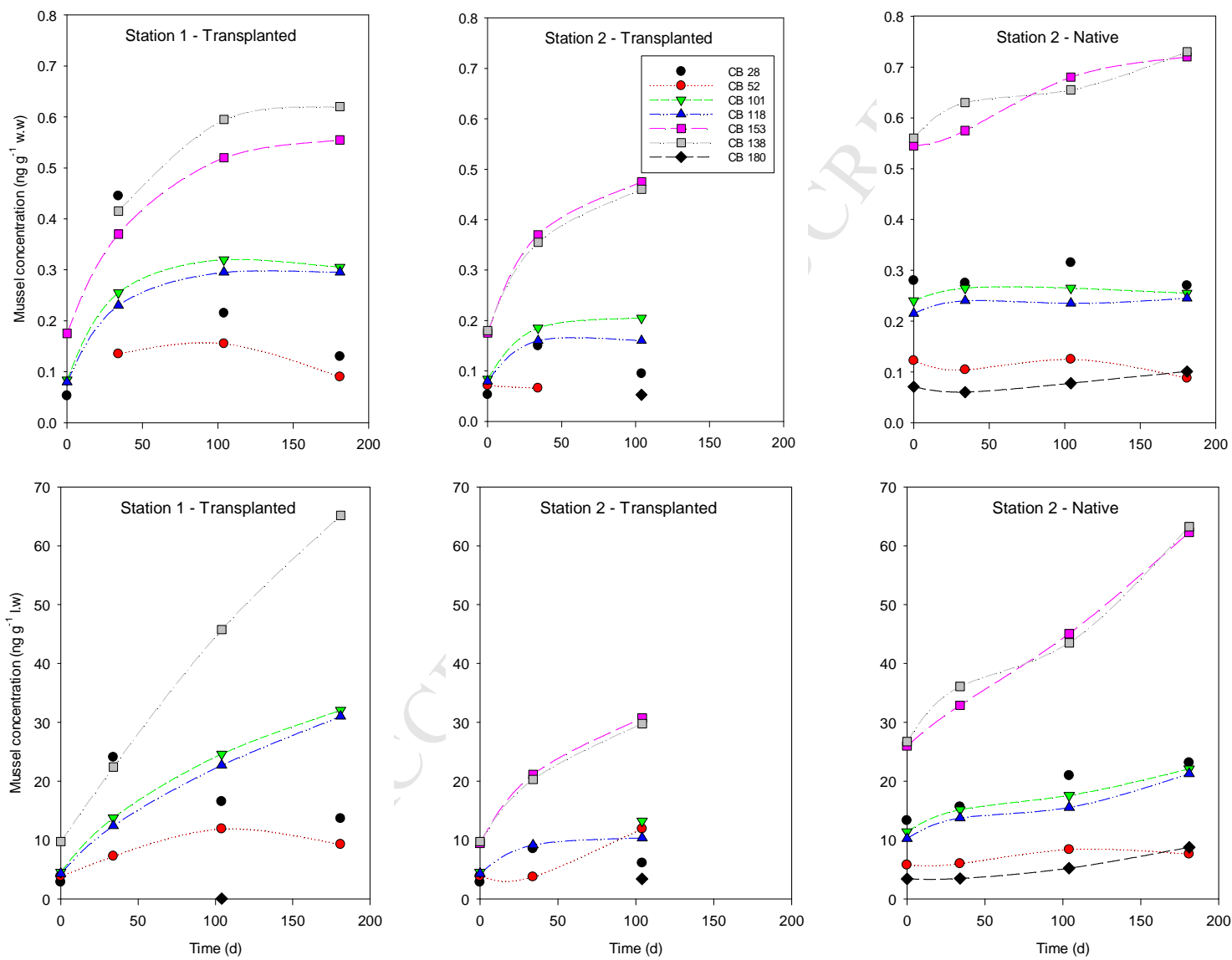
591 Figure 5



592

593

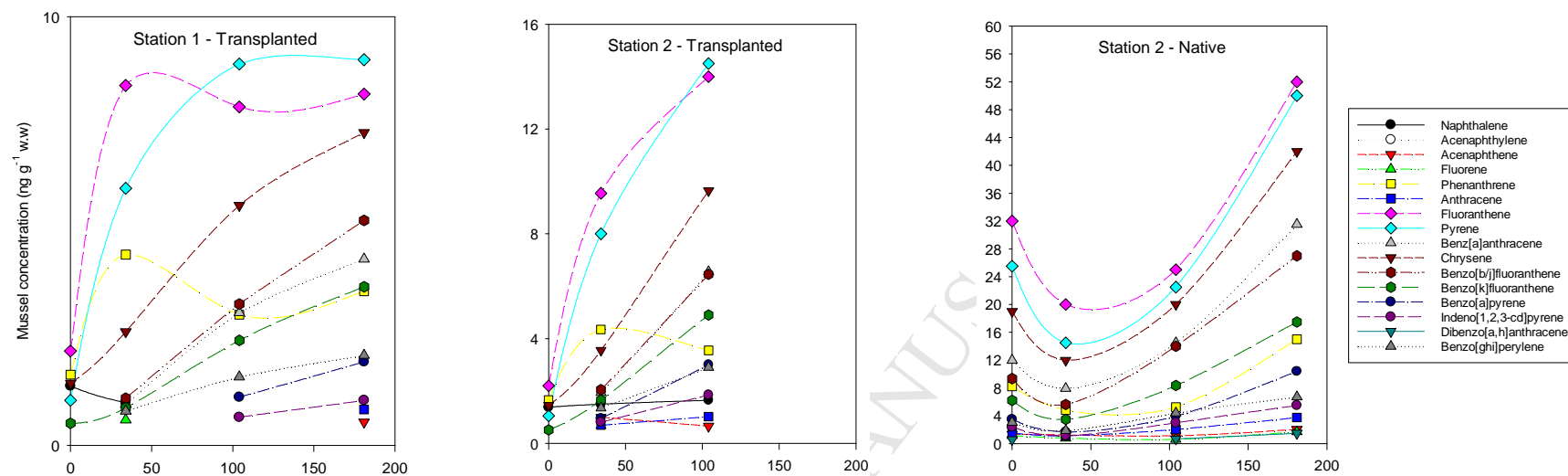
594 Figure 6



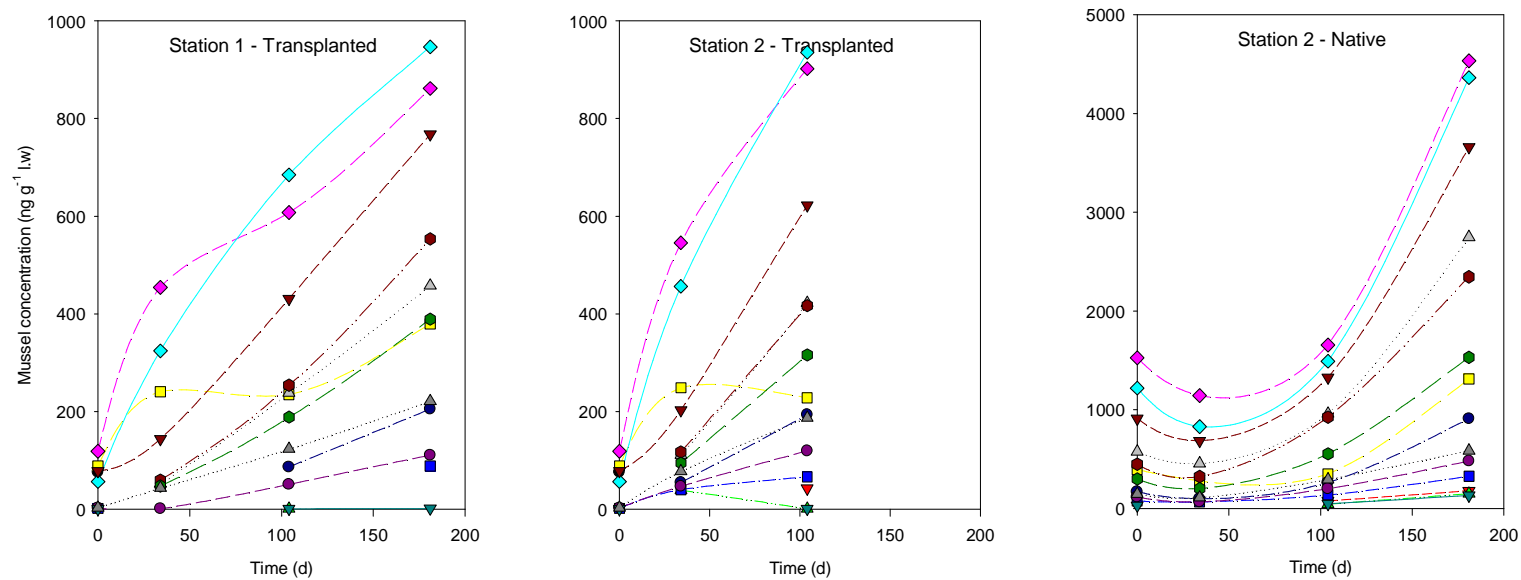
595

596

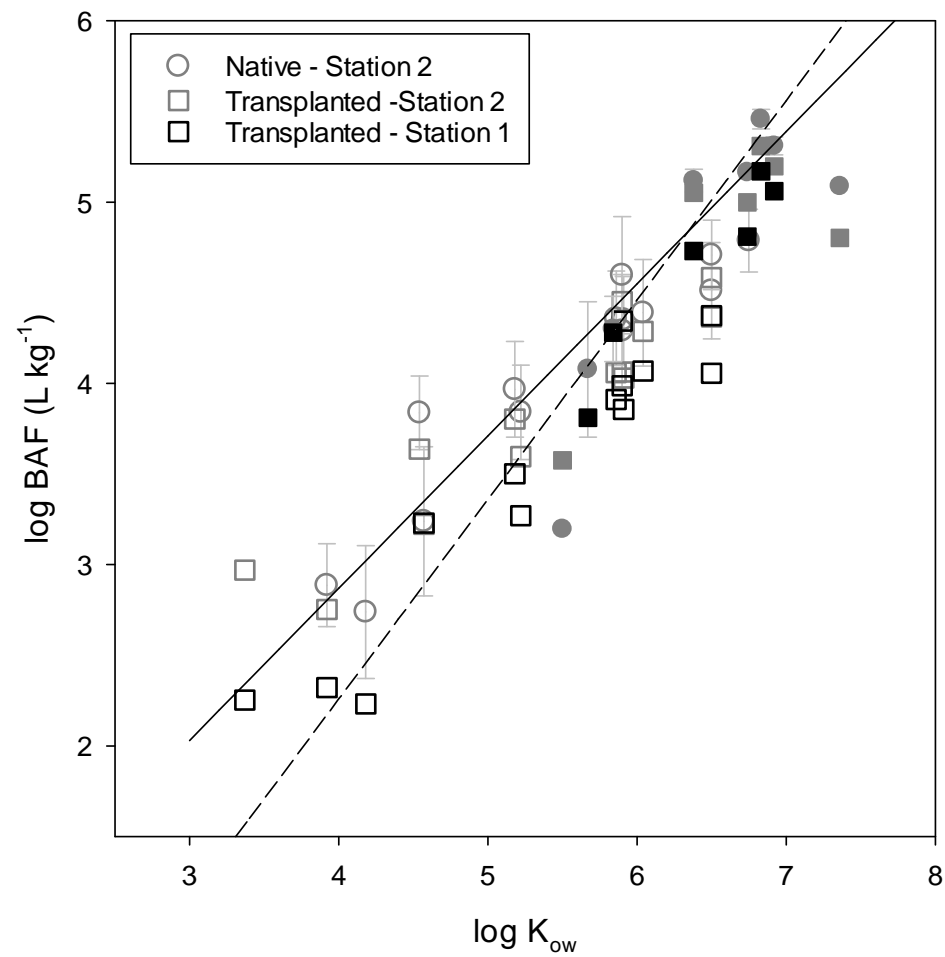
597 Figure 7



598

599
600

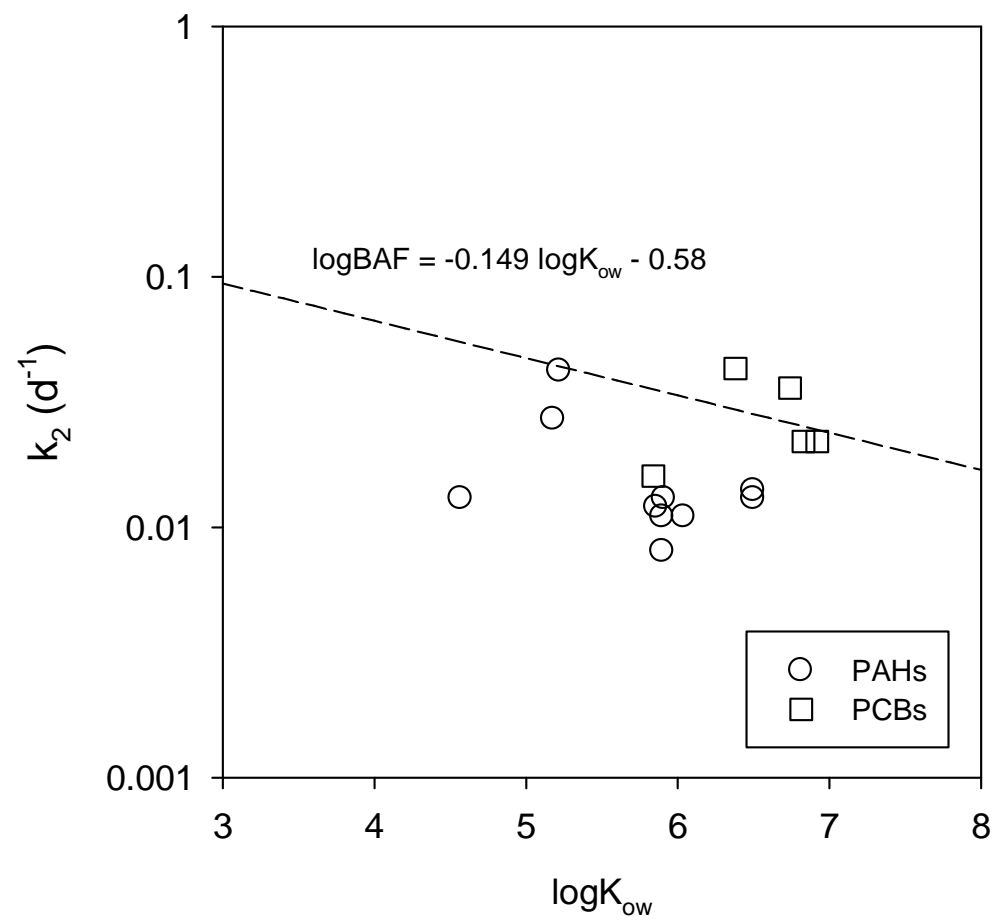
601 Figure 8



602

603

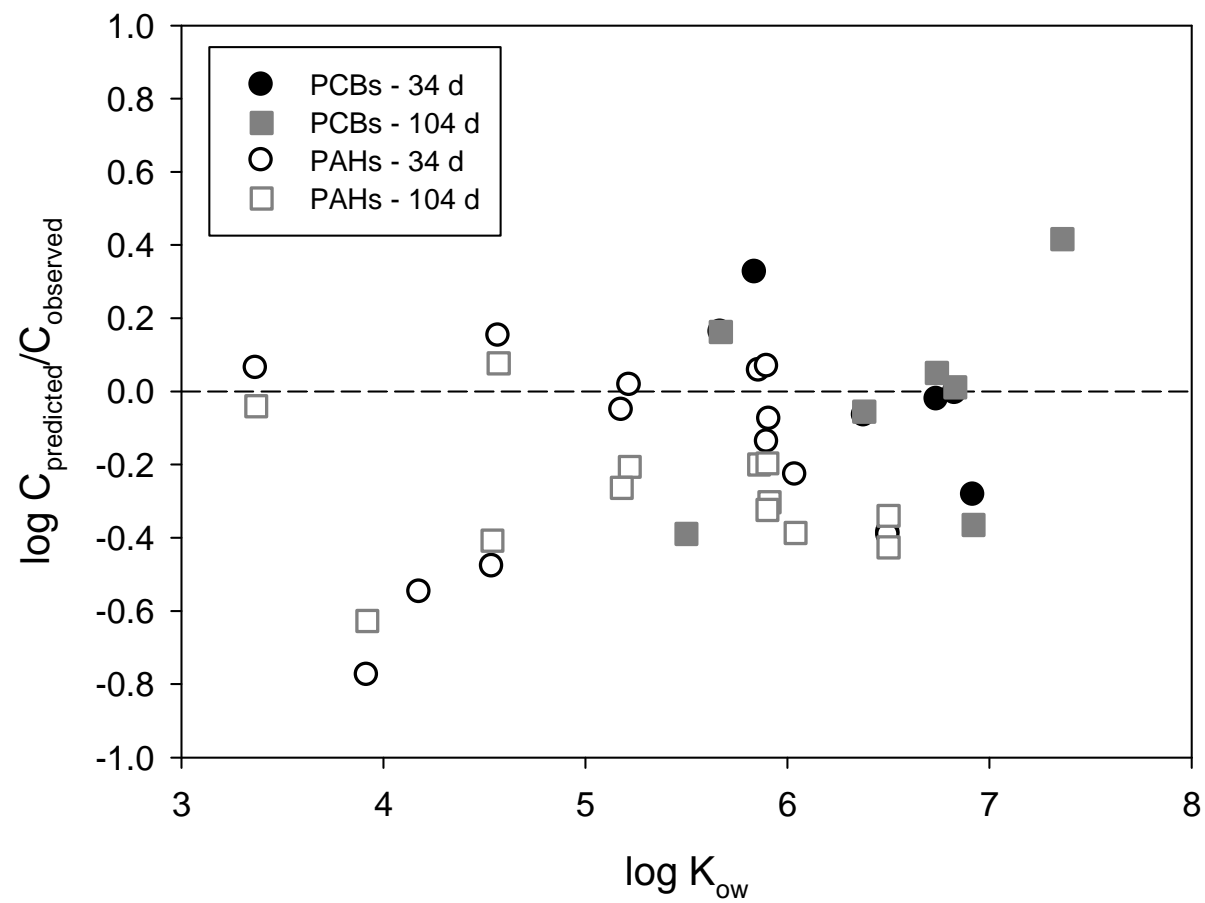
604 Figure 9



605

606

607 Figure 10



608

609

610

611

612 **Table 1: Contaminant concentrations (wet wt.) in deployed and native blue mussels in the Kristiansand harbor area at Station 1 (industrial site) and Station 2 (Svensholmen). All**
613 **concentrations are shown by the mean concentration of two replicate composite samples. For comparison, the rightmost columns show the 10 and 90 percentile concentration levels in**
614 ***M. edulis* from background and slightly impacted stations in the Norwegian coastal monitoring program, see Beyer et al. (this volume) for more details. Data shown in shaded boxes**
615 **are above the 90 percentile level.**

616

617 (Table 1 is uploaded separately as an excel file)

618

619

620

621

622 **Table 2. Blue mussel-water bioaccumulation factors (log BAF, wet wt.; L kg⁻¹) for trace elements measured in transplanted and native mussels (mg kg⁻¹ wet wt.) at two exposure sites**
 623 **based on DGT-labile concentrations in water (C_{DGT}; µg L⁻¹).**

	Mussel-water bioaccumulation factor (log BAF; L kg ⁻¹)							
	Station 1 (industrial site)			Station 2 (Svensholmen)				
	Transplanted			Transplanted		Native		
	34 d	104 d	181 d	34 d	104 d	July 2nd	Sept. 10th	Nov. 26th
Al	5.53	5.10	4.96	4.65	4.34	4.39	3.97	4.65
Pb	4.14	3.34	4.45	4.58	3.98	4.84	4.31	4.76
Fe	4.99	4.74	4.77	4.94	3.47	5.04	3.60	4.79
Cd	4.05	3.99	4.17	3.91	3.83	4.15	4.05	4.19
Ca	3.29	3.91	3.28	3.35	3.63	3.57	3.62	3.23
Cu	3.13	2.61	3.76	3.80	3.46	3.92	3.51	3.80
Co	2.77	2.75	2.97	3.43	3.24	3.61	3.06	3.65
Cr	4.15	4.19	4.06	4.00	3.85	4.28	4.13	4.00
Ni	2.30	2.50	2.33	2.77	2.68	2.89	2.74	2.93
Zn	4.27	3.95	4.16	4.25	4.08	4.14	4.06	4.33

624

625

626

627 **Table 3. Results from two parameter modelling of the uptake of PCBs into blue mussels exposed at Station 1 (industrial site).**

Parameters	BAF (L kg ⁻¹)*			Depuration rate, k ₂ (d ⁻¹)			R ²	log BAF	t _{90%} (d)**
	BAF	SE	P-value	k ₂	SE	p-value			
PCB31+28	6420		< 0.0001					3.81	
CB52	19139	4568	0.0086	0.016	0.01	0.153	0.80	4.28	144
CB101	53431	4004	< 0.0001	0.043	0.021	0.0930	0.88	4.73	54
CB118	64318	2888	< 0.0001	0.036	0.01	0.0141	0.96	4.81	64
CB153	113580	5708	< 0.0001	0.023	0.006	0.0120	0.96	5.06	105
CB138	147551	8398	<0.0001	0.022	0.005	0.0066	0.97	5.17	105

$\frac{C_m}{C_{Free}} = Y_0 + (BAF - Y_0)(1 - e^{-k_2 t})$
 with $Y_0 = C_{m(t=0)}/C_{free(34 d)}$
 * wet wt.-based bioaccumulation factors
 ** t_{90%} calculation based on the estimated depuration constant k₂ and that the mussel concentration for the contaminant of interest is negligible
 SE: standard error

628

629

630
631 **Table 4. Results from two-parameter modelling of the uptake of PAHs into blue mussels exposed at Station 1 (industrial site).**

Parameters	BAF (L kg ⁻¹)*			Depuration rate, k ₂ (d ⁻¹)			R ²	log BAF	t _{90%} (d)**
	BAF	SE	p-value	k ₂	SE	p-value			
Phenanthrene	1702	418	0.0097	0.012	0.008	0.2016	0.881	3.23	177
Fluoranthene	1854	158	< 0.0001	0.041	0.021	0.1144	0.872	3.27	54
Pyrene	3196	408	0.0005	0.027	0.015	0.1253	0.85	3.50	85
Benz[a]anthracene	7182	1663	0.0125	0.013	0.007	0.1523	0.803	3.86	177
Chrysene	8187	1708	0.0049	0.012	0.007	0.1601	0.909	3.91	192
Benzo[b/j]fluoranthene	9686	2868	0.0278	0.008	0.005	0.1445	0.899	3.99	288
Benzo[k]fluoranthene	22081	5772	0.0123	0.011	0.006	0.1323	0.871	4.34	209
Benzo[a]pyrene	11682	3468	0.078	0.011	0.009	0.3122	0.492	4.07	209
Indeno[1,2,3-cd]pyrene	11355	2897	0.0594	0.014	0.011	0.3298	0.35	4.06	164
Benzo[ghi]perylene	23660	2927	0.0013	0.013	0.004	0.0301	0.92	4.37	177

$\frac{C_m}{C_{Free}} = Y_0 + (BAF - Y_0)(1 - e^{-k_2 t})$
 with $Y_0 = C_{m(t=0)}/C_{free(34 d)}$
 * wet wt.-based bioaccumulation factors
 ** t_{90%} calculation based on the estimated depuration constant k₂ and that the mussel concentration for the contaminant of interest is negligible
 SE: standard error

632

633

634

635 **Table 5. Results of the linear regression of log BAF with logK_{ow} for PAHs and PCBs for native (Station 2, Svensholmen) and transplanted (Station 2, Svensholmen and Station 1,**
 636 **industrial site) mussels.**

			<i>n</i>	Slope			Intercept			R ²
				<i>a</i>	SE	<i>p</i> -value	Y ₀	SE	<i>p</i> -value	
PAHs	N	Station 2	14	0.692	0.066	< 0.0001	0.23	0.37	0.54	0.90
	T	Station 2	14	0.554	0.057	< 0.0001	0.88	0.31	0.016	0.90
	T	Station 1	13	0.738	0.069	< 0.0001	-0.43	0.37	0.27	0.92
PCBs	N	Station 2	8	1.02	0.24	0.0051	-1.8	1.5	0.28	0.71
	T	Station 2	7	0.86	0.25	0.018	-0.9	1.6	0.60	0.71
	T	Station 1	6	0.924	0.134	0.0023	-1.3	0.9	0.22	0.92

$\log BAF = a \log K_{ow} + Y_0$
 N: native mussels; T: transplanted mussels; SE: standard error

637

MERE_2017_201

Highlights

- Bioaccumulation of anthropogenic contaminants in deployed and native blue mussels during a six-month period was studied.
- Bioaccumulation factors for metals and organic contaminants were estimated.
- Differences in contaminant levels in transplanted and native mussels were observed.
- Significant confounding influence from seasonal factors on contaminant concentrations was observed.
- Standardization and harmonization of monitoring techniques that involve deployed and native blue mussels are needed.