

Credit author statement

Damien Réveillon : Conceptualization, Methodology, Supervision, Visualization, Writing – original draft, Funding acquisition.

Maxime Georges des Aulnois : Conceptualization, Methodology, Investigation, Visualization, Writing – original draft.

Véronique Savar : Methodology, Investigation, Visualization.

Elise Robert : Methodology, Investigation.

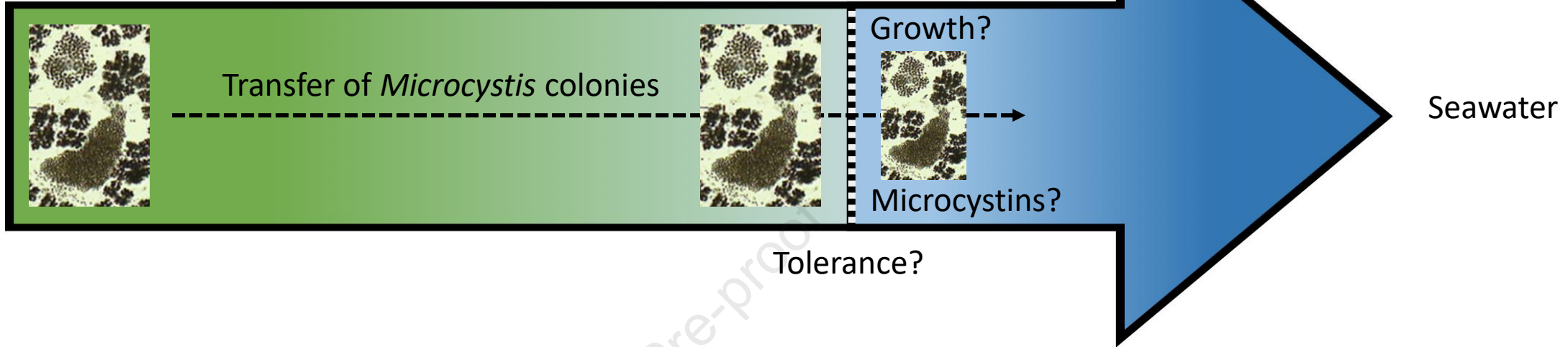
Amandine M. N. Caruana : Conceptualization, Supervision, Writing – review & editing, Funding acquisition.

Enora Briand : Conceptualization, Supervision, Writing – review & editing, Funding acquisition.

Myriam Bormans : Conceptualization, Supervision, Writing – review & editing, Funding acquisition.

Salinity gradient across the freshwater-to-marine continuum

Freshwater

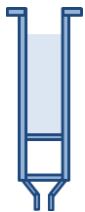


Seawater

Extraction of cyanotoxins

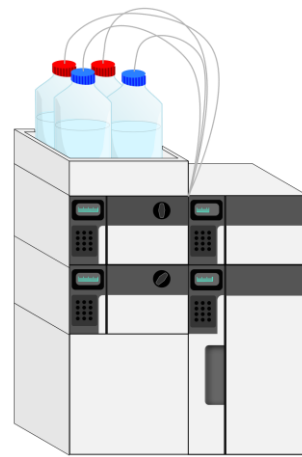


Intracellular (cells)

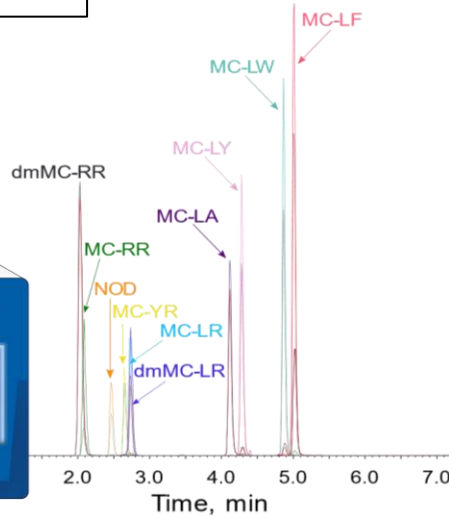
C18 vs. polymeric
cartridges for solid
phase extraction (SPE)

Extracellular (culture medium)

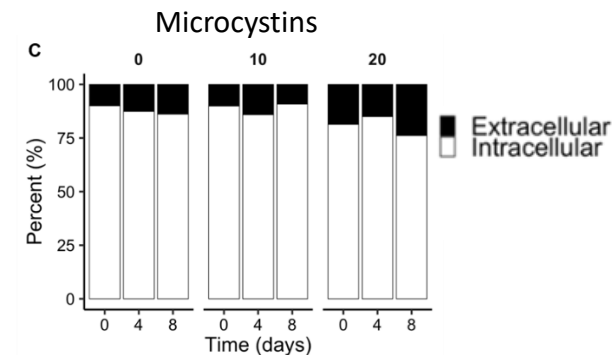
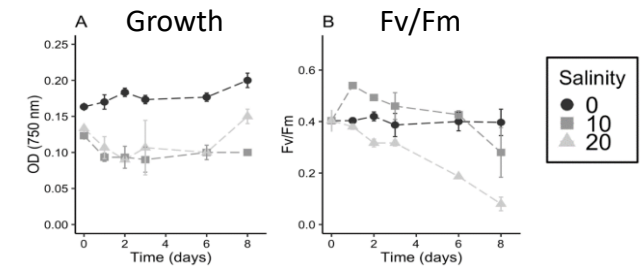
Analysis by LC-MS/MS



Liquid chromatography – tandem mass spectrometry

Characterization of the performance: recoveries and matrix effects
(corrective factors), sensitivity, accuracy, repeatability

Application to natural samples



1 **Extraction and analysis by liquid chromatography – tandem mass spectrometry**
2 **of intra- and extracellular microcystins and nodularin to study the fate of**
3 **cyanobacteria and cyanotoxins across the freshwater-marine continuum**

4

5 Damien Réveillon^{a,*}, Maxime Georges des Aulnois^a, Véronique Savar^a, Elise Robert^a,
6 Amandine M. N. Caruana^a, Enora Briand^a, Myriam Bormans^b

7 * Corresponding Author: damien.reveillon@ifremer.fr

8 ^a Ifremer, PHYTOX, F-44000 Nantes, France

9 ^b University of Rennes, CNRS, Ecobio UMR 6553, Rennes, France

10

11 **Highlights**

- 12 • Successful implementation of Solid Phase Extraction for extracellular
13 microcystins and nodularin in (salt)water samples.
- 14 • Accurate and repeatable methods for analysis of intracellular and extracellular
15 cyanotoxins using a corrective factor.
- 16 • Application of the analytical methods on natural *Microcystis* spp. colonies
17 subjected to a salinity shock.
- 18 • Colonies survived and toxins remained mainly intracellular, highlighting a
19 potential health risk for estuarine organisms.

20

21 **Abstract:**

22 The presence of microcystins (MCs) is increasingly being reported in coastal areas
23 worldwide. To provide reliable data regarding this emerging concern, reproducible and
24 accurate methods are required to quantify MCs in salt-containing samples. Herein, we
25 characterized methods of extraction and analysis by liquid chromatography coupled to
26 tandem mass spectrometry (LC-MS/MS) for nine MCs and one nodularin (NOD)
27 variants in both cyanobacteria (intracellular) and dissolved forms (extracellular).
28 Different approaches have been used to cope with salinity for the extraction of
29 dissolved MCs but none assessed solid phase extraction (SPE) so far. It was found

30 that salt had negligible effect on the SPE recovery of dissolved MCs using the C18
31 cartridge while an overestimation up to 67% was noted for some variants with a
32 polymeric sorbent. The limits of detection (LOD) and quantification (LOQ) were 1.0-22
33 and 5.5-124 pg on column for the intracellular toxins, while 0.05-0.81 and 0.13-2.4
34 ng/mL were obtained for dissolved toxins. Extraction recoveries were excellent for
35 intracellular (89-121%) and good to excellent for extracellular cyanotoxins (73-102%)
36 while matrix effects were considered neglectable (<12% for 16/20 toxin-matrix
37 combinations), except for the two MC-RR variants. The strategy based on the
38 application of a corrective factor to compensate for losses proved useful as the
39 accuracy was satisfactory (73-117% for intra- and 81-139% for extracellular
40 cyanotoxins, bias <10% for 46/60 conditions, with a few exceptions), with acceptable
41 precisions (intra- and inter-days variabilities <11%). We then applied this method on
42 natural colonies of *Microcystis* spp. subjected to a salt shock, mimicking their estuarine
43 transfer, in order to assess their survival and to quantify their toxins. The colonies of
44 *Microcystis* spp. had both their growth and photosynthetic activity impaired at salinities
45 from 10, while toxins remained mainly intracellular (>76%) even at salinity 20,
46 suggesting a potential health risk and contamination of estuarine organisms.

47 Key words: Coastal water, Microcystins, LC-MS/MS, Salinity, Solid Phase Extraction,
48 colonial *Microcystis*.

49

50 **1 Introduction**

51 Since the first report of microcystins (MCs) in American lakes in the 1930s (Bishop et
52 al., 1959), their presence has been documented on every continent, in aquatic
53 ecosystems from lakes to oceans as well as in terrestrial and extreme ecosystems
54 (Buratti et al., 2017; Cirés et al., 2017). Nearly 50 MC-producing cyanobacterial
55 species have been reported (Catherine et al., 2017), belonging mainly to common
56 freshwater blooming genera such as *Microcystis*, *Planktothrix*, *Aphanizomenon*,
57 *Dolichospermum* and *Oscillatoria* (Buratti et al., 2017). These non-ribosomally
58 synthesized heptapeptides share a common structure: cyclo-(D-Ala¹-X²-D-Masp³-
59 MeD³-Z⁴-Adda⁵-D-γ-Glu⁶-Mdha⁷) (Dittmann et al., 2013). Due to the numerous
60 combinations of amino acids, mainly at positions 2 and 4, and the occurrence of
61 demethylations, there is a huge diversity of MCs with more than 321 analogs reported

62 in CyanoMetDB (Jones et al., 2021), while new congeners are regularly being
63 discovered (e.g. Baliu-Rodriguez et al., 2022). Another family of at least 10 cyclic
64 pentapeptides (Jones et al., 2021) shares some similarities but was first detected in
65 the genus *Nodularia* and named nodularins accordingly (NODs).

66 Both MCs and NODs are hepatotoxins acting as specific inhibitors of eukaryotic protein
67 phosphatases (Bouaïcha et al., 2019; Mackintosh et al., 1990; Yoshizawa et al., 1990)
68 but they are also immunotoxic and show tumor-promoting, endocrine-disrupting and
69 oxidative DNA damage activities (Catherine et al., 2017). Consequently, MCs and
70 NODs are a threat for both humans and animals because of the global occurrence of
71 toxic cyanobacterial blooms (Huisman et al., 2018). The review by Svircev et al. (2019)
72 showed that almost 700 studies reported the detection of MCs in the environment
73 which accounted for 63% of the cyanotoxins reports published in peer-reviewed
74 journals until 2018. Besides, MCs were involved in most of the cyanotoxin-derived
75 poisoning events worldwide with 79 over the 189 cases reported in the literature up to
76 2018 (Svircev et al., 2019). Therefore, aquatic ecosystems and users are particularly
77 exposed while these cyanobacterial blooms are likely to increase and spread as a
78 consequence of human activities and climate changes (Paerl and Paul, 2012; Paerl et
79 al., 2018; Visser et al., 2016).

80 Proliferations of toxin-producing cyanobacteria also impact non-freshwater aquatic
81 ecosystems. In Europe, Baltic Sea is historically the main water ecosystem (i.e.
82 brackish) affected by MCs and NODs due to dense blooms of species of the genera
83 *Dolichospermum*, *Microcystis* and *Nodularia* (Brutemark et al., 2015; Mazur-Marzec et
84 al., 2010; Paldaviciene et al., 2015; Teikari et al., 2018). However, several studies
85 pointed out the occurrence of MCs in brackish and marine coastal waters as a
86 consequence of the physical transfer of cyanobacterial blooms and MCs along the
87 freshwater-to-marine continuum (Bormans et al., 2019; Gible et al., 2014; Lehman et
88 al., 2005; Robson and Hamilton, 2003). This phenomenon frequently recorded in North
89 America (Lehman et al., 2005; 2010; 2013; 2017; Moisander et al., 2009; Peacock et
90 al., 2018) has also been more recently observed in western European coastal waters,
91 both in Portugal (Churro et al., 2017; Lopes and Vasconcelos., 2011;) and France
92 (Bormans et al., 2019; Lance et al. 2021) where the presence of *Microcystis* spp. and
93 MCs in sediments was also reported (Bormans et al., 2020). This transfer results in a
94 salinity increase in the surrounding medium of cells with subsequent cell lysis when

95 salt tolerance is overpassed (Ross et al., 2019) and can ultimately lead to the release
96 of MCs in the water and to the accumulation of MCs in aquatic organisms e.g., shellfish,
97 fish and mammals (Bukaveckas et al., 2017; Lance et al., 2021; Lehman et al 2017;
98 Miller et al., 2010). In addition, some research groups consider that the contamination
99 of mussels may have originated from marine cyanobacteria in Greece (Kalaitzidou et
100 al., 2021; Vareli et al., 2013), although the MC-producing organisms remain to be
101 unequivocally discovered. Altogether, these observations call for the need to better
102 monitor the land-sea interface, where multiple sampling strategies are necessary
103 (Tatters et al., 2021).

104 Accordingly, the development of reliable and sensitive analytical methods is required
105 to accurately assess the presence of MCs in different matrices (Balest et al., 2016;
106 Massey et al., 2020; Turner et al., 2018), especially in the marine environment where
107 they are considered as emerging toxins (Howard et al., 2023; Paerl et al., 2018; Tatters
108 et al., 2017; 2021). Hitherto, most of the published methods focused on freshwater
109 ecosystems (e.g. lakes, rivers, reservoirs) for the extraction and quantification of MCs
110 and NODs in matrices such as natural and drinking waters, cyanobacteria, as well as
111 vegetal and animal tissues (Massey et al., 2020). However, seawater and more
112 generally salts can be responsible for “matrix effect” which can significantly affect
113 method performances (Bienvenu et al., 2017) from the sample preparation (extraction
114 recovery) up to the analysis (retention time (Bruns et al., 2022) or signal intensity
115 (Bruns et al., 2022; Munoz et al., 2017)) for LC-MS/MS methods, with potential strong
116 implications on the result accuracy if not properly assessed and controlled. This matrix
117 effect may also apply for other quantification methods such as enzyme linked
118 immunoassay (ELISA) or protein phosphatase inhibition assay (PPIA) and should be
119 further characterized as they have been used in the context of elevated salinity studies
120 (e.g. Lehman et al., 2017, Ross et al., 2019).

121 Nowadays, the most accurate and reliable methods to detect and quantify cyanotoxins
122 are based on liquid chromatography coupled to mass spectrometry (Beltran et al.,
123 2012; Benke et al., 2015; Haddad et al., 2019; Oehrle et al., 2010; Ortiz et al., 2017;
124 Romera-García et al., 2021; Turner et al., 2018). Therefore, we characterized a LC-
125 MS/MS method to quantify simultaneously nine MCs and one NOD analogs in
126 cyanobacteria and in water containing salts. Among the different sample preparation
127 available for environmental contaminants (Ali et al., 2019), a C18-SPE step for small

128 volume was implemented for the extraction of dissolved MCs (e.g. for laboratory study
129 purpose). Once characterized, this method was tested on natural bloom samples of
130 colonial *Microcystis* spp. exposed to two salinities (10 and 20), to mimic the transfer of
131 MC-producing freshwater cyanobacteria into estuarine waters and to assess the health
132 risk on estuarine organisms. Most previous experiments of salt-shock on *Microcystis*
133 species involved unicellular strains with no mucilage (Allakhverdiev et al., 2008;
134 Georges des Aulnois et al., 2020; Ross et al., 2019; Tonk et al., 2007) while colony
135 formation is suspected to protect the cells against environmental stress (including
136 salinity) (Bormans et al., 2023; Kehr and Dittmann, 2015). Therefore, we also
137 monitored the growth, cell lysis and MC intra- and extracellular concentrations of the
138 colonial *Microcystis* spp. in response to this salt increase.

139 **2 Material and methods**

140 2.1 Chemicals and reagents

141 Ultra-pure water was provided by a Milli-Q integral 3 system (Millipore). Methanol
142 (MeOH) and acetonitrile were of LC-MS grade (Honeywell) while formic acid >98%
143 purity (Fluka). Reference toxin standards (purity > 95%, MC-LR, MC-RR, MC-LY, MC-
144 LF, MC-LA, MC-YR, MC-LW, dmMC-RR, dmMC-LR and NOD) were purchased from
145 Novakits. Cyanobacterial growth media BG₁₁ and [BG₁₁ + 36 g/L NaCl] were prepared
146 according to Rippka et al. (1979) using chemicals from Merck.

147 2.2 Extraction of dissolved MCs: effect of salt on the recovery of two SPE cartridges

148 The effect of salt on SPE was assessed by comparing (n=3) recoveries in media with
149 and without salts (i.e. [BG₁₁ + 36 g/L NaCl] versus BG₁₁) spiked with toxin standards at
150 concentrations corresponding to 18 times the LOQs. Two types of SPE sorbents were
151 compared: a C18 (Bond Elut C18, 200 mg, 10 mL, Agilent) and a polymeric (Oasis
152 HLB, 200 mg, 6 mL, Waters). The same SPE procedure was applied for both:
153 conditioning with 3 mL of MeOH followed by 3 mL of water, then 15 mL of the spiked
154 matrix were loaded onto the cartridge before washing with 2.4 mL of MeOH/ water
155 (5:95, v/v) and elution with 4 mL of MeOH.

156 2.3 Characterization of the performance of the analytical method

157 All conditions were tested in triplicate, by spiking the blank matrices with the 10
158 reference toxin standards. All extracts were stored at -20 °C until LC-MS/MS analysis.

159 2.3.1 Extraction of intra- and extracellular toxins

160 The method was characterized for both intra- and extracellular toxins.
161 For the intracellular blank matrix, *M. aeruginosa* PCC 7806 *mcyB*⁻ (Dittman et al., 1997)
162 was used. This non-MC-producing mutant strain is available from the Pasteur Culture
163 collection of Cyanobacteria (PCC, <http://cyanobacteria.web.pasteur.fr/>). Cell pellets
164 (corresponding to 5 billion cells), obtained after centrifugation (4000 g, 15 min at room
165 temperature), were mechanically grinded in 1 mL of MeOH with 250 mg of glass beads
166 (0.15-0.25 mm; VWR) using a mixer mill (MM400; Retsch) for 30 min at 30 Hz
167 (Réveillon et al., 2014). Tubes were centrifuged at 13000 g and extracts were
168 ultrafiltered (Nanosep MF Bio-inert, 0.2 µm, Pall).
169 For the extracellular blank matrix, as recoveries were considered non-affected by salts
170 with the C18 sorbent, 15 mL of BG₁₁ medium were used and extracted with the Bond
171 Elut C18 cartridge, as aforementioned.

172 2.3.2 Liquid Chromatography-tandem Mass Spectrometry analysis (LC-MS/MS) of
173 MCs and NOD

174 Ultrafast liquid chromatography (Nexera; Shimadzu) coupled to a hybrid triple
175 quadrupole mass spectrometer (QTRAP 5500, Sciex) equipped with a TurboV
176 ionization source were used for LC-MS/MS analyses.

177 The chromatography was conducted using a Kinetex XB C18 column (100 x 2.1 mm;
178 2.6 µm; Phenomenex) equipped with a suited guard column and maintained at 25 °C.
179 The injection volume was 5 µL and the samples were kept at 4°C. The flow rate was
180 0.3 mL/min and the elution gradient with water (A) and acetonitrile (B) both containing
181 0.1% formic acid was 30-80% B (0 to 5 min), 80% B (5 to 6 min), and 30% B (6.5 to 11
182 min). The total runtime per sample was 11 min.

183 The MS/MS detection was performed using multiple reaction monitoring (MRM). The
184 electrospray ionization interface (ESI) was operated in positive mode using the
185 following settings: curtain gas 30 psi, ion spray 5000 V, temperature 300 °C, gas 1 and
186 2 set at 30 and 40 psi respectively, and entrance potential 10 V. Two transitions per
187 toxin were monitored (Table 1) with a dwell time of 40 ms for each. Quantification was
188 carried out using a 6-point external calibration curve (from 0.6 to 145 ng/mL) prepared
189 with the 10 standards (see Supplementary Fig. S1 for an example of chromatogram).
190 Data acquisition and processing were performed using Analyst 1.6.3 (Sciex).

191

192 Table 1 : Optimized transitions and mass spectrometer parameters for the MRM detection of
 193 MCs and NOD.

Analytes	MRM transitions (<i>m/z</i>)	DP (V)	CE (eV)	CXP (V)
dmMC-RR	512.8 > 135.0*; 103.0	121	35; 89	6; 16
MC-RR	520.1 > 135.2*; 200.1	76	45; 49	6; 12
MC-YR	1045.6 > 213.2*; 375.2	221	87; 71	12; 22
MC-LR	995.6 > 213.2*; 375.2	186	85; 70	14; 22
dmMC-LR	981.4 > 103.0*; 135.0	101	129; 117	12; 18
MC-LA	910.7 > 375.2*; 135.2	81	45; 91	8; 6
MC-LY	1002.6 > 135.2*; 375.3	106	93; 49	6; 8
MC-LW	1025.6 > 135.2*; 375.2	106	103; 53	12; 22
MC-LF	986.6 > 375.2*; 135.2	96	47; 93	8; 6
NOD	825.5 > 227.0*; 163.2	151	69; 67	12; 14

194 DP = declustering potential; CE = collision energy; CXP = cell exit potential

195 * quantitative MRM transition

196

197 2.3.3 Matrix effect, extraction recovery and corrective factor

198 Matrix effects (i.e. signal suppression or enhancement) on electrospray ionization of
 199 MCs and NOD were estimated by spiking the intra- and extracellular extracted matrices
 200 just before LC-MS/MS analysis at concentrations corresponding to 18 and 22 LOQs
 201 (i.e. intermediate level of concentration used to assess the performance of the method,
 202 see section 2.3.4). Results from the spiked extracts were compared to pure cyanotoxin
 203 standards in 100% MeOH to calculate the matrix effect as in Gosetti et al. (2010):

$$204 \text{ Matrix effect (\%)} = \frac{\text{Peak area in matrix}}{\text{Peak area in solvent}} \times 100$$

205 Extraction recoveries then corresponded to the deviation between the final
 206 concentration (i.e. with a matrix spiked before extraction) and the expected
 207 concentration:

$$208 \text{ Extraction recovery (\%)} = \frac{\text{Concentration}_{\text{matrix spiked before extraction}}}{\text{Concentration}_{\text{solvent spiked before analysis}}}$$

209 A corrective factor was finally calculated and applied to compensate the biases
 210 resulting from the sample preparation and the matrix effect:

$$211 \text{ Corrective factor (CF)} = \text{extraction recovery} \times \text{matrix effect}$$

212 2.3.4 Determination of limits of detection and quantification (LOD, LOQ)

213 The limits of detection (LOD) and quantification (LOQ) were determined for intracellular
214 and extracellular toxins. LODs were defined as the lowest concentrations giving a
215 mean (three consecutive injections) signal-to-noise (S/N) ratio of 3 for the qualifier
216 transition. LOQs were defined as the lowest concentrations giving a mean (for three
217 consecutive injections) signal-to-noise (S/N) ratio of 10 for the quantifier transition. For
218 both, S/N were calculated considering three times the standard deviation of the noise.

219 2.3.5 Performance of the method: accuracy, intra- and inter-day variabilities

220 The accuracy and intra- and inter-day variabilities of both analytical methods
221 (intracellular and extracellular) were then characterized at 3 levels of concentration
222 prepared in triplicate (4, 18, 80 LOQs and 4, 22, 125 LOQs respectively for intracellular
223 and extracellular fractions). The two blank matrices (i.e. toxin-free cell pellets of the
224 *mcvB* mutant strain of *M. aeruginosa* PCC 7806 and 15 mL of BG₁₁ medium extracted
225 with the Bond Elut C18 cartridge) were spiked before extraction and LC-MS/MS
226 analysis.

227 The accuracy was obtained after dividing by a corrective factor (see section 2.4.2).
228 Intra-day variability of the method (relative standard deviation, RSD) was evaluated
229 with three consecutive injections the same day while inter-day variability was evaluated
230 by comparing three injections every two days over one week (inter-batch, n=9).

231 2.4 Application to natural cyanobacterial bloom samples

232 Cyanobacterial bloom samples were collected in the freshwater reservoir of Pen Mur
233 in Brittany, France (47°33'44.4"N, 2°29'31.7"W). This reservoir is used for drinking
234 water and undergoes recurrent and intense cyanobacterial blooms dominated by MC-
235 producing *Microcystis* spp. (Bormans et al., 2019). This site is close to the ocean (< 10
236 km) and the water level in the reservoir is regulated by a flood barrier. In August 2018,
237 a surface scum sample (2 L) was collected and immediately brought back to the
238 laboratory to perform a salt shock experiment. Cyanobacterial identification was
239 performed under optical microscope, following the morphological descriptions of
240 Komárek and Komárková (2002).

241 A water sample (5 L) from the reservoir was also retrieved one meter below the surface
242 and used to prepare the media. After filtration on 1 µm glass microfiber filter (Whatman)
243 to remove cyanobacterial cells, it was mixed with natural filtered seawater (0.45 µm

244 cellulose filter; Whatman) in order to constitute two salt conditions corresponding to a
245 salinity of 10 and 20 as checked with a conductivity meter (Cond 3110 Set 1; WTW).

246 Triplicate 500 mL culture media at different salinities (0: control, 10 and 20) were
247 inoculated with the same volume of the surface scum sample and maintained for 8
248 days at $22 \pm 0.6^\circ\text{C}$ under a 12:12 h light:dark cycle using cool-white fluorescent tubes
249 (Philips) with $35 \mu\text{mol photons /m}^2/\text{s}$ illumination.

250 The optical density (OD, 750 nm) was used as a proxy of cell concentration and
251 monitored every day (Infinite® M200 plate reader; Tecan). The maximum quantum
252 efficiency of the photosystem II (F_v/F_m) was used as a proxy of the physiological status
253 of the cyanobacterial cells and monitored daily on 15 min dark-adapted samples (630
254 nm, no DCMU addition and the basal fluorescence was not subtracted) (Ogawa et al.,
255 2017) using an Aquapen-C fluorimeter (Photon Systems Instruments).

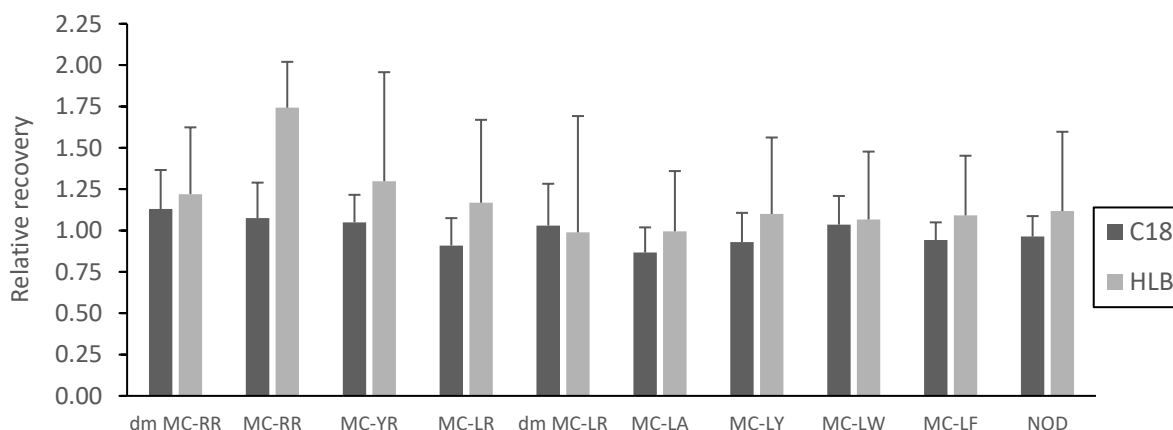
256 The cultures were sampled every two days by centrifugation (15 mL, 4000 g 15 min at
257 room temperature) and both pellets and supernatants were stored at -20°C before
258 extraction and LC-MS/MS analysis. The quantification of intracellular and extracellular
259 MCs was obtained by applying the appropriate corrective factor.

260

261 **3 Results**

262 3.1 Effect of salt on SPE recovery

263 In order to assess the effect of salt, the SPE recoveries obtained with the salt-
264 containing medium (i.e., BG₁₁ + 36 g/L NaCl) were compared with those obtained with
265 the salt-free medium (i.e., BG₁₁ as reference) and expressed as a relative recovery
266 (Fig. 1). The C18 cartridge gave similar and more repeatable relative recoveries with
267 and without salt for all toxins (i.e. 0.87-1.13) while only 6 toxins were in that range for
268 the HLB cartridge. In addition, the relative recovery of 3 MCs was markedly affected
269 with the HLB cartridge (1.22, 1.30 and 1.74 for dmMC-RR, MC-YR and MC-RR) for
270 which an increase in recovery due to the salt effect was stronger.



271
 272 Figure 1: Relative recoveries between [BG₁₁ + 36 g/L NaCl] and BG₁₁ media for each MC
 273 analog and the two SPE cartridges tested (C18 and HLB) (n = 3). Error bars denote the
 274 standard deviation calculated based on the standard approach of error propagation in
 275 calculated ratios (Holmes and Buhr, 2007).

276 As a result, the C18 cartridge was selected as it showed less salt-derived matrix effects
 277 compared to HLB, under the conditions tested.

278 3.2 Characterization of the methods for intra- and extracellular MCs and NOD

279 3.2.1 LODs and LOQs

280 Limits of detection and quantification for intracellular and extracellular matrices are
 281 provided in Table S1. The LODs and LOQs ranged from 1.0 to 22 pg and from 5.5 to
 282 124 pg (on column), in the cyanobacterial cell matrix and from 0.59 to 11 pg and from
 283 1.8 to 34 pg (on column) in the extracellular matrix, respectively. For the latter, the
 284 method detection limit (MDL) and method quantification limit (MQL) ranged from 0.04
 285 to 0.81 ng/mL and from 0.13 to 2.4 ng/mL, respectively (i.e. for a 15 mL sample
 286 concentrated by a factor 3.75 by SPE, after application of the corrective factors defined
 287 in Table 2). There was a ca. 20-fold difference between the lowest (for dmMC-RR) and
 288 the highest (for MC-LR or MC-YR) LOD and LOQ, for both the intracellular and
 289 extracellular matrices.

290 3.2.2 Extraction recovery, matrix effect and corrective factor

291 Extraction recovery, matrix effect on ionization and the resulting corrective factor (CF)
 292 were evaluated for the intermediate spiking level for both intracellular and extracellular
 293 toxins (Table 2).

294

295 Table 2: Extraction recovery, matrix effect and corrective factor (CF) for each toxin in
 296 the intracellular and extracellular matrices (mean \pm SD, n = 3).

Variant	Intracellular			Extracellular		
	Extraction recovery \pm SD (%)	Matrix effect \pm SD (%)	CF	Extraction recovery \pm SD (%)	Matrix effect \pm SD (%)	CF
dmMC-RR	89 \pm 4	132 \pm 4	1.2	30 \pm 2	228 \pm 4.6	0.68
MC-RR	90 \pm 3	111 \pm 3	0.99	43 \pm 8	152 \pm 11	0.65
MC-YR	116 \pm 5	101 \pm 1	1.2	73 \pm 1	103 \pm 1.0	0.75
MC-LR	121 \pm 4	100 \pm 1	1.2	77 \pm 2	102 \pm 1.0	0.79
dmMC-LR	108 \pm 6	96 \pm 2	1.0	83 \pm 3	102 \pm 1.0	0.85
MC-LA	96 \pm 2	98 \pm 4	0.94	102 \pm 5	98 \pm 2.9	1.0
MC-LY	93 \pm 4	104 \pm 4	0.97	99 \pm 4	100 \pm 1.0	0.99
MC-LW	95 \pm 3	112 \pm 3	1.1	81 \pm 3	103 \pm 2.1	0.83
MC-LF	100 \pm 3	120 \pm 4	1.2	77 \pm 3	100 \pm 1.0	0.77
NOD	96 \pm 4	98 \pm 3	0.94	95 \pm 1	101 \pm 2.0	0.96

310
 311 Overall, extraction recoveries ranged between 89 \pm 4% and 121 \pm 4% for the
 312 intracellular toxins and between 73 \pm 1% and 102 \pm 5% for the extracellular toxins.
 313 Only, dmMC-RR and MC-RR showed poor SPE extraction recoveries of 30 \pm 2% and
 314 43 \pm 8% respectively.

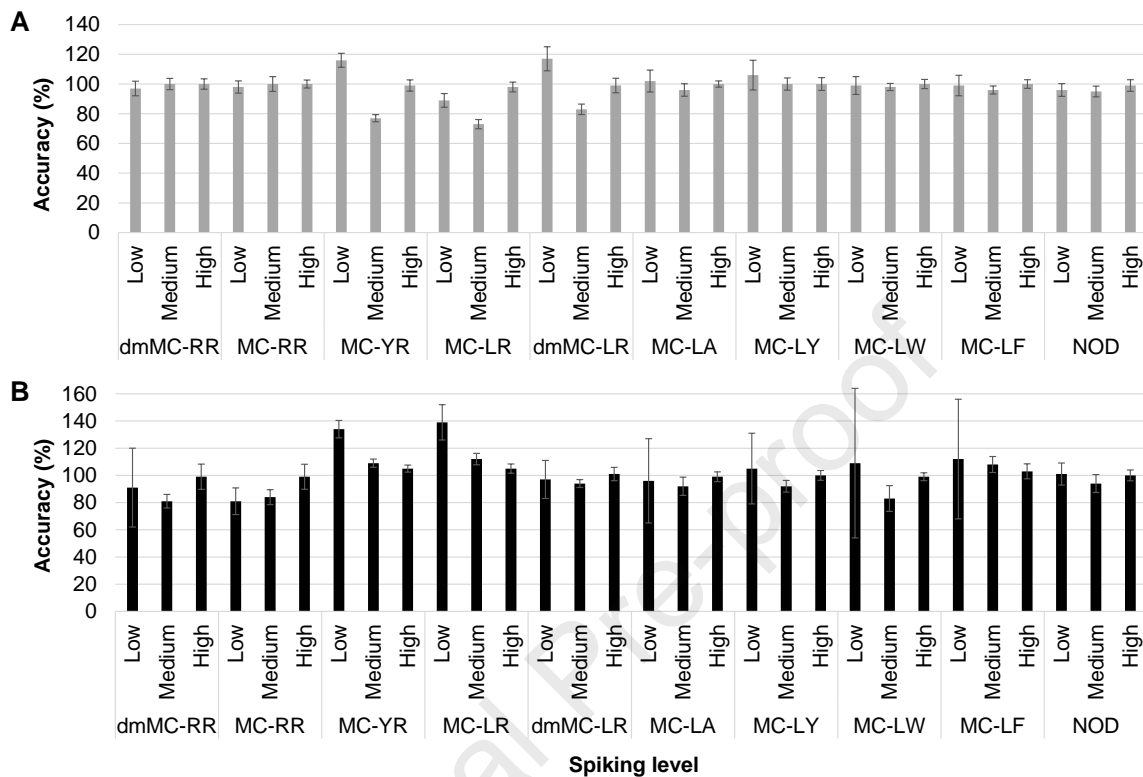
315 Generally, low matrix effects were observed for the great majority of MC variants,
 316 except for MC-LF (120 \pm 3.6%) and dmMC-RR (132 \pm 4.0%) for the intracellular matrix
 317 and for dmMC-RR (152 \pm 11%) and MC-RR (228 \pm 4.6%) for the extracellular matrix.

318 As a result, the corrective factors were weak for the intracellular toxins (0.94-1.2) while
 319 they were higher for the extracellular toxins (0.65-1.0), reflecting the balance between
 320 lower extraction recoveries and strong matrix effect, especially for MC-RR and dmMC-
 321 RR.

323 3.2.3 Accuracy for intra- and extracellular toxins

324 The accuracy was calculated for each MC variant and NOD at each of the three levels
 325 of the spiked, extracted and analyzed matrices (Fig. 2, Table S2). For the intracellular
 326 matrix, accuracies ranged between 73 \pm 3.1% and 117 \pm 8.1%. An excellent
 327 performance close to 100% (i.e. 95-102%) for the three spiking levels was noted for 7

328 variants (dmMC-RR, MC-RR, -LA, -LY, -LW, -LF and NOD). For MC-YR, MC-LR and
 329 dmMC-LR, the accuracy range was wider, between 77 – 116%, 73 – 98% and 83 –
 330 117% depending on the spiking level considered (Fig. 2 A).



331
 332 Figure 2: Accuracies for intracellular (A) and extracellular toxins (B) for each spiking level
 333 (mean \pm SD, n=3). Low, Medium and High spiking levels corresponded to 4, 18 and 80 LOQs
 334 for the intracellular matrix and 4, 22 and 125 LOQs for the extracellular matrix.

335 For the extracellular matrix, accuracies ranged between $81 \pm 9.8\%$ and $139 \pm 13\%$
 336 (Fig. 2 B). The performance was considered excellent (i.e. accuracy of 90-110%) for
 337 22/30 conditions (combination of 10 variants and 3 spiking levels) while for 3 variants
 338 at the lowest level of spiking, an overestimation was noted (i.e. accuracy of 112-139%
 339 for MC-YR, MC-LR and MC-LF), and some underestimation were also observed for
 340 the other conditions (e.g. for MC-RR at the low and medium levels of spiking).

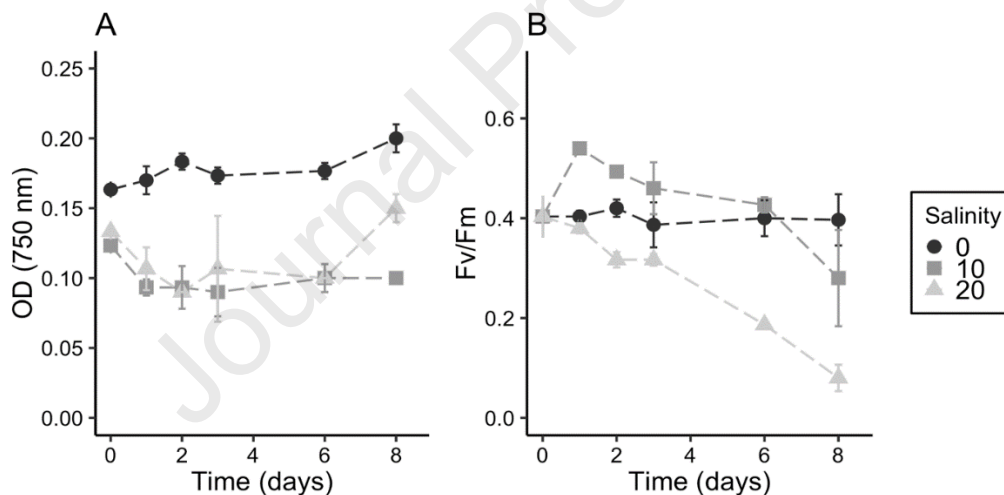
341 3.2.4 Intra- and inter-day variabilities

342 Intra- and inter-day variabilities were good to excellent for both intracellular and
 343 extracellular toxins for all variants at medium and high spiking levels (RSD of 0.3-11%,
 344 Table S3). For the low level of spiking, a wider inter-day variability was obtained for
 345 dmMC-RR, MC-LA, -LY and -LW (RSD of 25-51%).

346 3.3 Salt shock experiment on natural colonies of *Microcystis* spp.

347 In the natural bloom sample, the genus *Microcystis* dominated the phytoplankton
 348 community (>95%) and the morpho-species *M. aeruginosa* was dominant while some
 349 colonies of *M. botrys* and *M. viridis* were also noted. Over the 8-day experiment, OD
 350 increased only in the control condition from 0.16 ± 0.01 to 0.20 ± 0.01 (Fig. 3A). At a
 351 salinity of 10, after an initial decline from 0.12 ± 0.01 on day 1, OD remained stable
 352 around 0.10 ± 0.01 . The same trend was obtained at a salinity of 20 except between
 353 days 6 and 8 where the OD increased from 0.10 ± 0.02 to 0.15 ± 0.01 (Fig. 3A). The
 354 maximum quantum yield of photosystem II (Fv/Fm) remained stable in the control
 355 condition between 0.39 ± 0.03 and 0.42 ± 0.01 (Fig. 3B). At a salinity of 10, Fv/Fm
 356 values increased from 0.40 ± 0.04 to 0.54 ± 0.01 on day 1, before decreasing steadily
 357 to 0.28 ± 0.11 at the end of the experiment. At a salinity of 20, a linear decrease from
 358 0.40 ± 0.04 to 0.08 ± 0.03 was observed (Fig. 3B).

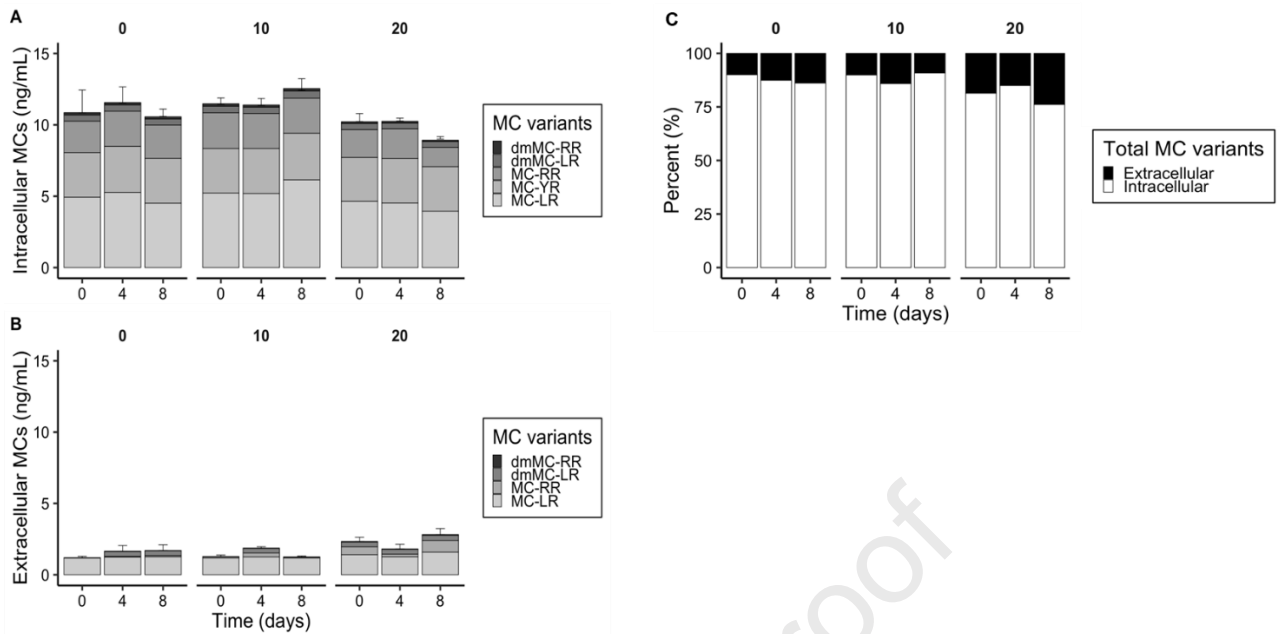
359



360

361 Figure 3: Effect of different salinity conditions on optical density (A) and on the maximum
 362 quantum yield of photosystem II (Fv/Fm; B) of natural bloom samples (mean \pm SD, n = 3).

363 In the cyanobacterial scum, 5 MCs were detected (MC-LR, MC-YR, MC-RR, dmMC-
 364 LR and dmMC-RR) (Fig. 4). Irrespective of salinity and time, the proportion of the
 365 different variants was 45/31/21/2/1% (MC-LR/-YR/-RR/dmMC-LR/dmMC-RR).



366

367 Figure 4: (A) Intracellular and (B) extracellular MC profiles (mean \pm SD, n=3; SD was calculated
 368 on the total of each MC variant quantified) and (C) proportion (%) of intra- and extracellular
 369 toxins of natural bloom samples submitted to three salt treatments (0, 10 and 20) and over
 370 time (4, 8 days).

371 The total amounts of intracellular MCs were in a close range and remained stable over
 372 time, irrespective of salinity (from 9.0 ± 0.18 to 12 ± 0.81 ng/mL; Fig. 4A). In the
 373 extracellular fraction, only four MCs were quantified as MC-YR was detected but below
 374 the LOQ (Fig. 4B). At salinities 0 and 10, the extracellular fraction represented 10 -
 375 14% and 9 - 14% of total MCs over time (Fig. 4C) while at a salinity of 20, this proportion
 376 was slightly higher (15 - 24%). Overall, the proportion of extracellular MCs tended to
 377 increase with salinity and time (Fig. 4C).

378

379 4 Discussion

380 This study aimed to characterize a method for the simultaneous extraction and
 381 quantification of nine MCs and one NOD in cyanobacterial cells and as dissolved in
 382 both fresh- and salt-containing waters, corresponding to intra and extracellular MCs.
 383 First, an SPE procedure designed for laboratory studies on relatively small volume
 384 samples was implemented for the dissolved toxins. Afterwards, the LC-MS/MS method
 385 was characterized for the detection and quantification of these ten cyanotoxins. Finally,
 386 the characterized methods (extraction and analysis) were applied to test the effect of
 387 a salinity shock on *Microcystis* spp. colonies and on intra- and extracellular MC

388 contents by exposing a natural bloom sample to a sudden salt increase, to mimic a
389 transfer across the freshwater-marine continuum.

390 Extractions by SPE are routinely used for the analysis of dissolved MCs in freshwater,
391 allowing for concentration of MCs before LC-MS/MS analysis (Mashile and
392 Nomngongo, 2016) while direct injections are also possible when sensitivity is not an
393 issue (e.g. Mekebri et al., 2009; Tran et al., 2020; Turner et al., 2018). By contrast, a
394 direct injection of seawater samples in mass spectrometers is not recommended due
395 to the high concentration of salts possibly resulting in interferences during the
396 electrospray ionization process (Hemmati et al., 2019). Such a phenomenon has been
397 reported for the analysis of total MCs in surface waters but due to the oxidation
398 process, with a severe decrease as high as 75% of the signal (Munoz et al., 2017). As
399 matrix effects can significantly affect method performance, they have to be evaluated
400 first, and then reduced as much as possible, either by appropriate sample preparation
401 or correction or a combination of both. While SPATT (Solid Phase Adsorption Toxin
402 Tracking) using HP20 resin and SALLE (salting-out assisted liquid-liquid extraction)
403 were successfully implemented for the extraction of dissolved MCs from saltwaters
404 (Hemmati et al., 2019; Kudela et al., 2011), we chose to assess the effectiveness of
405 SPE, by comparing the effect of salt on the recovery of the two types of cartridges
406 mainly used for MCs, namely C18 and HLB (Mashile and Nomngongo, 2016). Unlike
407 for HLB cartridge, recoveries with the C18 cartridge were not affected by salt addition
408 (i.e. relative recovery with and without salt between 0.87 and 1.13 for the 10 toxins
409 considered). Therefore, the C18-SPE appeared suitable to both remove salts and
410 concentrate extracellular MCs and NOD. Concerning intracellular MCs, we considered
411 that salinity would not affect the extraction, based on previous data (Georges des
412 Aulnois et al., 2019). Finally, the methods' performances to extract and analyze
413 intracellular and extracellular MCs were assessed, using these two extraction
414 protocols.

415 While aqueous methanol gave better recoveries for intracellular MCs extracted from
416 cyanobacteria in previous studies (Fastner et al., 1998; Romera- García et al., 2021;
417 Turner et al., 2018), here recoveries obtained with pure methanol using a mixer mill
418 and glass beads were excellent in the spiked *Microcystis* matrix (89-121%). This
419 suggests that methanol associated with an efficient cell lysis (Réveillon et al., 2014) is

420 appropriate to extract the 9 MCs and NOD from wet cyanobacteria, despite their
421 different polarities.

422 Recoveries of dissolved cyanotoxins extracted by C18-SPE from BG₁₁ medium were
423 good to excellent (73-102%) except for the two more hydrophilic and doubly-arginated
424 variants (30 and 43% for dmMC-RR and MC-RR). Despite differences in protocols,
425 these recoveries are in agreement with previous studies that used SPE (e.g. 48-157%
426 with C18-SPE in Mekebri et al. (2009) or 70-107% with HLB-SPE in Kaloudis et al.
427 (2013)) or on-line C18-SPE (94-102% for Fayad et al. (2015)) or other techniques such
428 as SALLE (53-100%, Hemmati et al., 2019) to extract dissolved cyanotoxins from water
429 samples, although some studies reported better recoveries for MC-RR (e.g. 100% in
430 Fayad et al. (2015) or total recovery of 76-143% in Mekebri et al. (2009)). In our case,
431 this low recovery was counterbalanced by the unusual strong matrix-derived signal
432 enhancement observed for the two -RR variants, leading to an acceptable total
433 recovery (i.e. extraction recovery and matrix effect) of 65-68%. Ion suppressions are
434 generally reported for MCs, but strong signal enhancements have already been
435 observed for some phycotoxins, such as DTX1 (>300% in mussel matrix, Zendong et
436 al., 2015). Except for these two conditions, matrix effects observed here were
437 considered negligible (i.e. lower than 12% for 16 out of the 20 toxin-matrix
438 combinations). Assessing matrix effects is essential, as it is analyte-, matrix-, sample
439 preparation- and instrument-dependent (Zendong et al., 2015) and can have a
440 significant impact on the signal intensity. For examples, Turner et al. (2018) observed
441 different matrix effects between water, cyanobacteria, shellfish and algal supplement
442 tablet, while Hemmati et al. (2017) or Tran et al. (2020) noted strong signal suppression
443 of 80% for MC-RR and NOD extracted by SALLE from hypersaline water or ca. 55%
444 vs. 18% for MC-RR and dmMC-RR extracted by HLB-SPE from freshwater vs. after
445 direct injection.

446 While different strategies have been successfully implemented to provide accurate
447 results, such as matrix-matched calibration curves (Munoz et al., 2017) or the use of
448 internal standards (Haddad et al., 2019; Tran et al., 2020), we tried to compensate for
449 losses (i.e. due to extraction recoveries and residual matrix effects) by applying a
450 predefined corrective factor for each analyte in each matrix while relying on external
451 calibration curves in solvent for the quantification. This strategy provided an excellent
452 accuracy for most variants and spiking levels for the intracellular and extracellular

453 toxins (<10% bias for 26/30 and 22/30 combinations, respectively), with only two
454 conditions for intracellular and two for extracellular toxins showing a bias >20% (MC-
455 YR and MC-LR at low or intermediate levels of spiking). Similar level of performance
456 were reported when using a matrix-matched calibration curve for water samples
457 (Munoz et al., 2017), making this approach a suitable alternative, but not as accurate
458 as methods using isotopically labelled MCs as internal standards (Haddad et al., 2019;
459 Tran et al., 2020). Still, we obtained highly repeatable recoveries and matrix effects
460 (standard deviations <8 and 11%, respectively), which is as good as or even better
461 than Haddad et al. (2019) and Tran et al. (2020) despite their use of internal standards.
462 Both intra- and inter-day variabilities were excellent for intracellular cyanotoxins (RSD
463 <10%) suggesting a high precision within and between batches. The variabilities were
464 slightly higher for extracellular cyanotoxins, especially for the inter-day comparison and
465 for the lowest spiking level, but still acceptable for most conditions (RSD <17% for
466 26/30 combinations). Overall, these levels of precision are in line with previous studies
467 for cyanobacterial cells (Turner et al., 2018) and waters (Kaloudis et al., 2013; Romera-
468 García et al., 2021; Turner et al., 2018) or when considering similar levels of spiking
469 (Ortiz et al., 2017).

470 The LC-MS/MS methods characterized here showed LOD and LOQ in the range of
471 1.0-22 pg and 5.5-124 pg (on column) in the intracellular matrix (*M. aeruginosa* cells)
472 and 0.59-11 pg and 1.8-34 pg (on column) in the extracellular matrix (BG11 medium).
473 These information are not systematically reported unfortunately, while they would ease
474 the comparison between methods and mass spectrometer sensitivity, but when
475 comparing to the available or inferred data, our sensitivities seem similar (for four
476 variants) or higher than Turner et al. (2018) by a factor 9-22 in cyanobacterial cells (i.e.
477 when comparing the ng spiked), but ca. 7 to 40 times better than Haddad et al. (2019)
478 (20-80 and 70-280 pg) for dissolved toxins (i.e. in a spiked blank water sample).
479 Although not directly comparable, Tran et al. (2020) reported excellent instrumental
480 LOD and LOQ (i.e. based on standards) of 0.06-2.5 and 0.20-8.0 pg. When considering
481 the factor of SPE concentration (i.e. 3.75) and after application of the corrective factors,
482 method detection and quantification limits (MDL and MQL) were 0.04-0.81 ng/mL and
483 0.13-2.4 ng/mL. Except for the two least sensitive MC variants (MC-YR and -LR), these
484 values are in the higher range of what is generally reported in the literature when using
485 pre-concentration by SPE (Kaloudis et al., 2013; Haddad et al., 2019; Munoz et al.,

486 2017; Tran et al., 2020; and see the table ESI1 in Romera et al. (2021) for other
487 examples) or other technique (Hemmati et al., 2019), as low to hundreds ng/L
488 sensitivities are common. However, the most sensitive methods used very different
489 concentration factors (3.75 here vs. 27-1000 in the above-mentioned studies) so a
490 direct comparison should be avoided. The MDL and MQL obtained here are in good
491 compliance with existing guidelines or sanitary threshold values for drinking and
492 recreational waters (Chorus and Welker, 2021), although the emerging risk caused by
493 MCs across the freshwater-marine continuum and in coastal waters has not been
494 properly addressed yet.

495 Still, our analytical methods could be improved. It should be noted that the optimization
496 of the SPE protocols was beyond the scope of the current study, however, a better
497 reduction of matrix effect may be pursued, for example by modifying the cleaning step,
498 as the wash volume was found to be a critical parameter for the appropriate desalting
499 in Munoz et al. (2017). Similarly, extraction recovery could be increased, especially for
500 the more polar variants (i.e. MC-RR, MC-YR and dmMC-RR) and future considerations
501 should concern the pH of the samples (Haddad et al., 2019; Tran et al., 2020), the
502 solvent(s) for elution, the type of filter (Fayad et al., 2015; Ortiz et al., 2017) or the
503 losses associated with adsorptions as depicted by Altaner et al. (2017). Also, more MC
504 variants could be included (e.g. 12 MCs in Tran et al. (2020) and 17 MCs in Pekar et
505 al. (2016) vs. 9 variants here) to better quantify the chemodiversity. In addition, we
506 could increase the factor of concentration for dissolved cyanotoxins, to improve the
507 method sensitivity but this can significantly increase the matrix effects by concentrating
508 both toxins and interferences (Tran et al., 2020). Ultimately, the accuracy would be
509 further increased by using internal standards. To this end, many studies have been
510 using NOD, a strategy questioned by Munoz et al. (2017) as affecting negatively the
511 accuracy compared to matrix-matched calibration curves. On the opposite, the use of
512 isotopically labelled MCs as internal standards gave excellent performance (Haddad
513 et al., 2019; Tran et al., 2020).

514 Overall, the corrective factors satisfactorily improved the accuracies of the methods
515 while the degree of variability (i.e. extractions for both intra and extracellular toxins,
516 matrix effects), precision (intra/inter-day) and the sensitivity obtained here were fit for
517 purpose.

518 The sample preparations and LC-MS/MS method were then applied on a natural
519 *Microcystis* spp. bloom sample mainly composed of *M. aeruginosa* colonies and
520 subjected to two salt treatments in the laboratory. Our objective was to assess the
521 potential growth and induced cell lysis of the colonies, together with detecting and
522 quantifying the MC analogs. A sudden increase in salinity inhibited the growth of
523 *Microcystis* spp., associated with a decrease in the maximum quantum efficiency of
524 photosystem II, and an increase in the proportion of extracellular toxins, in a
525 concentration dependent manner. The MC profile (5 congeners: MC-LR, MC-YR, MC-
526 RR, dmMC-LR and dmMC-RR) of the natural bloom remained similar between salinity
527 conditions, as previously observed with isolated strains (Georges des Aulnois et al.,
528 2019). Similar growth inhibition and/or photosynthetic activity responses to salt shocks
529 have been previously reported on unicellular strains (Georges des Aulnois et al., 2020;
530 Ross et al., 2019; Tonk et al., 2007) and colonies (Orr et al., 2004; Bormans et al.,
531 2023) of *Microcystis* spp. The range of salt tolerance of *Microcystis* spp. corresponds
532 to the mesohaline condition (e.g. 0-18) (Georges des Aulnois et al., 2019) with higher
533 thresholds for colonial forms, as observed here. Indeed, even at salinity 20, the optical
534 density remained high and toxins mainly intracellular, most likely due to the colonial
535 form of the natural bloom and the role of mucilage (Bormans et al., 2023; Reigner et
536 al., 2023; Xiao et al., 2019). Taken together, these results suggested that *Microcystis*
537 spp. produced no particular MC analogs in response to salt shock, that natural blooms
538 could survive in high salinity estuaries while releasing more toxins into the surrounding
539 environment, the latter indicating the beginning of cell lysis though (Ross et al., 2019).

540 The present results suggest that natural blooms of *Microcystis* spp. could persist during
541 their transfer to the estuarine zones of the Pen Mur outflow even at salinities higher
542 than mesohaline values, while extracellular MCs could increase. These hypotheses
543 are corroborated by both laboratory experiment and field monitoring that reported the
544 presence of intact *Microcystis* spp. colonies and detection of similar MC profile in
545 coastal water and sediment in this area (Bormans et al., 2019; 2020).

546 This study pointed out the necessity to characterize analytical methods for several MC
547 analogs based on seawater matrices to better assess the actual risk for animal and
548 human health. In addition to their application in the research field, the development
549 and implementation of these analytical methods for monitoring MCs and NOD in fresh
550 and coastal waters meet a need for assistance tools to decision intended for health

551 and environmental risk managers. This is of particular importance as the transfer of
552 freshwater cyanotoxins across the freshwater-marine continuum (Paerl et al., 2018) is
553 increasing, as attested by numerous reports of MCs in coastal areas and diverse
554 matrices (e.g., dissolved in water, bloom samples and animal and vegetal tissues)
555 (Peacock et al., 2018; Preece et al., 2017).

Journal Pre-proof

556 Acknowledgements: This research was funded by IFREMER and the Regional Council
557 of the region des Pays de la Loire.

558 Conflicts of Interest: The authors declare no conflict of interest.

559 References

560 Ali, I., Suhail, M., Alharbi, O.M.L., Hussain, I., 2019. Advances in sample preparation
561 in chromatography for organic environmental pollutants analyses. *Journal of Liquid*
562 *Chromatography & Related Technologies* 42, 137-160.
563 [10.1080/10826076.2019.1579739](https://doi.org/10.1080/10826076.2019.1579739).

564 Altaner, S., Puddick, J., Wood, S.A., Dietrich, D.R., 2017. Adsorption of Ten
565 Microcystin Congeners to Common Laboratory-Ware Is Solvent and Surface
566 Dependent. *Toxins* 9, 129. <https://doi.org/10.3390/toxins9040129>.

567 Balest L., Murgolo S., Sciancalepore L., Montemurro P., Abis P.P., Pastore C.,
568 Mascolo G., 2016. Ultra-trace levels analysis of microcystins and nodularin in
569 surface water by on-line solid-phase extraction with high-performance liquid
570 chromatography tandem mass spectrometry. *Analytical and Bioanalytical*
571 *Chemistry* 408, 4063–4071. <https://doi.org/10.1007/s00216-016-9495-y>.

572 Baliu-Rodriguez, D., Peraino, N.J., Premathilaka, S.H., Birbeck, J.A., Baliu-Rodriguez,
573 T., Westrick, J.A., Isailovic, D., 2022. Identification of Novel Microcystins Using
574 High-Resolution MS and MSn with Python Code. *Environmental science &*
575 *technology*. <https://doi.org/10.1021/acs.est.1c04296>.

576 Beltrán E., Ibáñez M., Sancho J.V., Hernández F., 2012. Determination of six
577 microcystins and nodularin in surface and drinking waters by on-line solid phase
578 extraction-ultra high pressure liquid chromatography tandem mass spectrometry.
579 *Journal of Chromatography A* 1266, 61–68.
580 <https://doi.org/10.1016/j.chroma.2012.10.017>.

581 Benke P.I., Vinay Kumar M.C.S., Pan D., Swarup S., 2015. A mass spectrometry-
582 based unique fragment approach for the identification of microcystins. *Analyst* 140,
583 1198–1206. <https://doi.org/10.1039/c4an01702a>.

584 Bienvenu, J.-F., Provencher, G., Bélanger, P., Bérubé, R., Dumas, P., Gagné, S.,
585 Gaudreau, É., Fleury, N., 2017. Standardized Procedure for the Simultaneous
586 Determination of the Matrix Effect, Recovery, Process Efficiency, and Internal
587 Standard Association. *Analytical Chemistry* 89, 7560-7568.
588 <https://doi.org/10.1021/acs.analchem.7b01383>.

589 Bishop C.T., Anet E.F., Gorham P.R., 1959. Isolation and identification of the fast-
590 death factor in *Microcystis aeruginosa* NRC-1. *Canadian Journal of Biochemistry*
591 *and Physiology* 37, 453–471. <https://doi.org/10.1139/o59-047>.

592 Bormans M., Amzil Z., Mineaud E., Brient L., Savar V., Robert E., Lance E., 2019.
593 Demonstrated transfer of cyanobacteria and cyanotoxins along a freshwater-
594 marine continuum in France. *Harmful Algae* 87, 101639.
595 <https://doi.org/10.1016/j.hal.2019.101639>.

596 Bormans, M., Legrand, B., Waisbord, N., Briand, E., 2023. Morphological and

- 597 physiological impacts of salinity on colonial strains of the cyanobacteria *Microcystis*
598 *aeruginosa*. *MicrobiologyOpen* 12, e1367. <https://doi.org/10.1002/mbo3.1367>.
- 599 Bormans M., Savar V., Legrand B., Mineaud E., Robert E., Lance E., Amzil Z., 2020.
600 Cyanobacteria and cyanotoxins in estuarine water and sediment. *Aquatic Ecology*
601 54, 625–640. <https://doi.org/10.1007/s10452-020-09764-y>.
- 602 Bouaïcha N., Miles C.O., Beach D.G., Labidi Z., Djabri A., Benayache N.Y., Nguyen-
603 Quang T., 2019. Structural Diversity, Characterization and Toxicology of
604 Microcystins. *Toxins* 11, 714. <https://doi.org/10.3390/toxins11120714>.
- 605 Bruns, S., Wienhausen, G., Scholz-Böttcher, B., Wilkes, H., 2022. Simultaneous
606 quantification of all B vitamins and selected biosynthetic precursors in seawater
607 and bacteria by means of different mass spectrometric approaches. *Anal Bioanal*
608 *Chem* 414, 7839-7854. <https://doi.org/10.1007/s00216-022-04317-8>.
- 609 Brutemark A., Vandelannoote A, Engström-Öst J., Suikkanen S., 2015. A less saline
610 Baltic Sea promotes cyanobacterial growth, hampers intracellular microcystin
611 production, and leads to strain-specific differences in allelopathy. *PLOS ONE* 10,
612 1–15. <https://doi.org/10.1371/journal.pone.0128904>.
- 613 Bukaveckas P.A., Lesutienė J., Gasiūnaitė Z.R., Ložys L., Olenina I., Pilkaitytė R.,
614 Pūtys Z., Tassone S., Wood J., 2017. Microcystin in aquatic food webs of the Baltic
615 and Chesapeake Bay regions. *Estuarine, Coastal and Shelf Science* 191, 50–59.
616 <https://doi.org/10.1016/j.ecss.2017.04.016>.
- 617 Buratti F.M., Manganelli M., Vichi S., Stefanelli M., Scardala S., Testai E., Funari E.,
618 2017. Cyanotoxins: producing organisms, occurrence, toxicity, mechanism of
619 action and human health toxicological risk evaluation. *Archives of Toxicology*, 1–
620 82. <https://doi.org/10.1007/s00204-016-1913-6>.
- 621 Catherine A., Bernard C., Spoo L., Bruno M., 2017. Microcystins and Nodularins.
622 <https://doi.org/10.1002/9781119068761.ch11>.
- 623 Chorus, I., Welker, M., 2001. Exposure to cyanotoxins Understanding it and short-term
624 interventions to prevent it, *Toxic Cyanobacteria in Water: A Guide to Their Public*
625 *Health Consequences, Monitoring and Management* (2nd ed.). CRC Press.
626 <https://doi.org/10.1201/9781003081449>.
- 627 Churro C., Azevedo J., Vasconcelos V., Silva A., 2017. Detection of a *Planktothrix*
628 *agardhii* bloom in Portuguese marine coastalwaters. *Toxins* 9, 1–13.
629 <https://doi.org/10.3390/toxins9120391>.
- 630 Cirés S., Casero M., Quesada A., 2017. Toxicity at the Edge of Life: A Review on
631 Cyanobacterial Toxins from Extreme Environments. *Marine Drugs* 15, 233.
632 <https://doi.org/10.3390/md15070233>.
- 633 Dittmann E., Fewer D.P., Neilan B.A., 2013. Cyanobacterial toxins: Biosynthetic routes
634 and evolutionary roots. *FEMS Microbiology Reviews* 37, 23–43.
635 <https://doi.org/10.1111/j.1574-6976.2012.12000.x>.
- 636 Dittmann, E., Neilan, B.A., Erhard, M., von Döhren, H., and Börner, T., 1997.
637 Insertional mutagenesis of a peptide synthetase gene that is responsible for
638 hepatotoxin production in the cyanobacterium *Microcystis aeruginosa* PCC 7806.
639 *Mol Microbiol* 26, 779–787.

- 640 Fastner, J., Flieger, I., Neumann, U., 1998. Optimised extraction of microcystins from
641 field samples — a comparison of different solvents and procedures. *Water*
642 *Research* 32, 3177-3181. [https://doi.org/10.1016/S0043-1354\(98\)00073-6](https://doi.org/10.1016/S0043-1354(98)00073-6).
- 643 Fayad, P.B., Roy-Lachapelle, A., Duy, S.V., Prévost, M., Sauvé, S., 2015. On-line
644 solid-phase extraction coupled to liquid chromatography tandem mass
645 spectrometry for the analysis of cyanotoxins in algal blooms. *Toxicon* 108, 167-175.
646 <https://doi.org/10.1016/j.toxicon.2015.10.010>.
- 647 Georges des Aulnois, M., Réveillon, D., Robert, E., Caruana, A., Briand, E., Guljamow,
648 A., Dittmann, E., Amzil, Z., Bormans, M., 2020. Salt Shock Responses of
649 *Microcystis* Revealed through Physiological, Transcript, and Metabolomic
650 Analyses. *Toxins* 12, 192. <https://doi.org/10.3390/toxins12030192>
- 651 Georges des Aulnois M., Roux P., Caruana A., Réveillon D., Briand E., Hervé F., Savar
652 V., Bormans M., Amzil Z., 2019. Physiological and Metabolic Responses of
653 Freshwater and Brackish-Water Strains of *Microcystis aeruginosa* Acclimated to a
654 Salinity Gradient: Insight into Salt Tolerance. *Applied and Environmental*
655 *Microbiology* 85, 1–15. <https://doi.org/10.1128/AEM.01614-19>.
- 656 Gible C.M., Kudela R.M., 2014. Detection of persistent microcystin toxins at the land-
657 sea interface in Monterey Bay, California. *Harmful Algae* 39, 146–153.
658 <https://doi.org/10.1016/j.hal.2014.07.004>.
- 659 Gosetti F., Mazzucco E., Zampieri, D., Gennaro, M.C., 2010. Signal
660 suppression/enhancement in high-performance liquid chromatography tandem
661 mass spectrometry. *J. Chromatogr. A* 1217, 3929–3937.
- 662 Haddad S.P., Bobbitt J.M., Taylor R.B., Lovin L.M., Conkle J.L., Chambliss C.K.,
663 Brooks B.W., 2019. Determination of microcystins, nodularin, anatoxin-a,
664 cylindrospermopsin, and saxitoxin in water and fish tissue using isotope dilution
665 liquid chromatography tandem mass spectrometry. *Journal of Chromatography A*
666 1599, 66–74. <https://doi.org/10.1016/j.chroma.2019.03.066>.
- 667 Hemmati, M., Tejada-Casado, C., Lara, F.J., García-Campaña, A.M., Rajabi, M., del
668 Olmo-Iruela, M., 2019. Monitoring of cyanotoxins in water from hypersaline
669 microalgae colonies by ultra high performance liquid chromatography with diode
670 array and tandem mass spectrometry detection following salting-out liquid-liquid
671 extraction. *Journal of Chromatography A* 1608, 460409.
672 <https://doi.org/10.1016/j.chroma.2019.460409>.
- 673 Holmes, D.T., Buhr, K.A., 2007. Error propagation in calculated ratios. *Clinical*
674 *Biochemistry* 40, 728-734. <https://doi.org/10.1016/j.clinbiochem.2006.12.014>.
- 675 Howard, M.D.A., Smith, J., Caron, D.A., Kudela, R.M., Loftin, K., Hayashi, K., Fadness,
676 R., Fricke, S., Kann, J., Roethler, M., Tatters, A., Theroux, S., 2023. Integrative
677 monitoring strategy for marine and freshwater harmful algal blooms and toxins
678 across the freshwater-to-marine continuum. *Integrated environmental assessment*
679 *and management* 19, 586-604. <https://doi.org/10.1002/ieam.4651>.
- 680 Huisman J., Codd G.A., Paerl H.W., Ibelings B.W., Verspagen J.M.H, Visser P.M.,
681 2018. Cyanobacterial blooms. *Nature Reviews Microbiology* 16, 471–483.
682 <https://doi.org/10.1038/s41579-018-0040-1>.
- 683 Jones, M.R., Pinto, E., Torres, M.A., Dörr, F., Mazur-Marzec, H., Szubert, K.,

- 684 Tartaglione, L., Dell'Aversano, C., Miles, C.O., Beach, D.G., McCarron, P.,
685 Sivonen, K., Fewer, D.P., Jokela, J., Janssen, E.M.L., 2021. CyanoMetDB, a
686 comprehensive public database of secondary metabolites from cyanobacteria.
687 Water Research 196, 117017. <https://doi.org/10.1016/j.watres.2021.117017>.
- 688 Kalaitzidou, M.P., Nannou, C.I., Lambropoulou, D.A., Papageorgiou, K.V.,
689 Theodoridis, A.M., Economou, V.K., Giantsis, I.A., Angelidis, P.G., Kritas, S.K.,
690 Petridou, E.J., 2021. First report of detection of microcystins in farmed
691 mediterranean mussels *Mytilus galloprovincialis* in Thermaikos gulf in Greece.
692 Journal of biological research (Thessalonike, Greece) 28, 8.
693 <https://doi.org/10.1186/s40709-021-00139-4>.
- 694 Kaloudis T., Zervou S.K., Tsimeli T., Triantis T.M., Fotiou T., Hiskia A., 2013.
695 Determination of microcystins and nodularin (cyanobacterial toxins) in water by LC–
696 MS/MS. Monitoring of Lake Marathonas, a water reservoir of Athens, Greece.
697 Journal of Hazardous Materials 263, 105–115.
698 <https://doi.org/10.1016/j.jhazmat.2013.07.036>.
- 699 Kehr J-C and Dittmann, E., 2015. Biosynthesis and function of extracellular glycans in
700 cyanobacteria. Life (5): 164-180. doi:10.3390/life5010164
- 701 Komárek J., Komárková J., 2002. Review of the European *Microcystis* morphospecies
702 (Cyanoprokaryotes) from nature. Czech Phycology 2, 1–24.
- 703 Kudela, R.M., 2011. Characterization and deployment of Solid Phase Adsorption Toxin
704 Tracking (SPATT) resin for monitoring of microcystins in fresh and saltwater.
705 Harmful Algae 11, 117-125. <https://doi.org/10.1016/j.hal.2011.08.006>.
- 706 Lance E., Lepoutre A., Savar V., Robert E., Bormans M., Amzil Z., 2021. In situ use of
707 bivalves and passive samplers to reveal water contamination by microcystins along
708 a freshwater-marine continuum in France. Water Research 204, 117620 (10p.)
709 <https://doi.org/10.1016/j.watres.2021.117620>.
- 710 Lehman P.W., Kurobe T., Lesmeister S., Baxa D., Tung A., The S.J., 2017. Impacts of
711 the 2014 severe drought on the *Microcystis* bloom in San Francisco Estuary.
712 Harmful Algae 63, 94–108. <https://doi.org/10.1016/j.hal.2017.01.011>.
- 713 Lehman P.W., Marr K., Boyer G.L., Acuna S., The S.J., 2013. Long-term trends and
714 causal factors associated with *Microcystis* abundance and toxicity in San Francisco
715 Estuary and implications for climate change impacts. Hydrobiologia 718, 141–158.
716 <https://doi.org/10.1007/s10750-013-1612-8>.
- 717 Lehman P.W., The S.J., Boyer G.L., Nobriga M.L., Bass E., Hogle C., 2010. Initial
718 impacts of *Microcystis aeruginosa* blooms on the aquatic food web in the San
719 Francisco Estuary. Hydrobiologia 637, 229–248. <https://doi.org/10.1007/s10750-009-9999-y>.
- 721 Lehman P.W., Boyer G., Hall C., Waller S., Gehrts K., 2005. Distribution and toxicity
722 of a new colonial *Microcystis aeruginosa* bloom in the San Francisco Bay Estuary
723 California. Hydrobiologia 541, 87–99. <https://doi.org/10.1007/s10750-004-4670-0>.
- 724 Lopes V.R., Vasconcelos V., 2011. Planktonic and benthic cyanobacteria of European
725 brackish waters: A perspective on estuaries and brackish seas. European Journal
726 of Phycology 46, 292–304. <https://doi.org/10.1080/09670262.2011.602429>.
- 727 MacKintosh C., Beattie K.A., Klumpp S., Cohen P., Codd G.A., 1990. Cyanobacterial

- 728 microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A
729 from both mammals and higher plants. *FEBS Letters* 264, 187–192.
730 [https://doi.org/10.1016/0014-5793\(90\)80245-E](https://doi.org/10.1016/0014-5793(90)80245-E).
- 731 Mashile G.P., Nomngongo P.N., 2016. Recent Application of Solid Phase Based
732 Techniques for Extraction and Preconcentration of Cyanotoxins in Environmental
733 Matrices. *Critical Reviews in Analytical Chemistry* 8347, 1–8.
734 <https://doi.org/10.1080/10408347.2016.1225255>.
- 735 Massey I.Y., Wu P., Wei J., Luo J., Ding P., Wei H., Yang F., 2020. A Mini-Review on
736 Detection Methods of Microcystins. *Toxins* 12, 641.
737 <https://doi.org/10.3390/toxins12100641>.
- 738 Mazur-Marzec H., Forycka K., Kobos J., Pliński M., Browarczyk-Matusiak G., 2010.
739 Morphological, genetic, chemical and ecophysiological characterisation of two
740 *Microcystis aeruginosa* isolates from the Vistula Lagoon, southern Baltic.
741 *Oceanologia* 52, 127–146. <https://doi.org/10.5697/oc.52-1.127>.
- 742 Mekebri A., Blondina G.J., Crane B.D., 2009. Method validation of microcystins in
743 water and tissue by enhanced liquid chromatography tandem mass spectrometry.
744 *Journal of Chromatography A* 1216, 3147–3155.
745 <https://doi.org/10.1016/j.chroma.2009.01.095>.
- 746 Miller M.A., Kudela R.M., Mekebri A., Crane D., Oates S.C., Tinker M.T., Staedler M.,
747 Miller W.A., Toy-Choutka S., Dominik C., Hardin D., Langlois G., Murray M., Ward
748 K., Jessup D.A., 2010. Evidence for a novel marine harmful algal bloom:
749 Cyanotoxin (microcystin) transfer from land to sea otters. *PLoS ONE* 5, 1–11.
750 <https://doi.org/10.1371/journal.pone.0012576>.
- 751 Moisander P.H., Lehman P.W., Ochiai M., Corum S., 2009. Diversity of *Microcystis*
752 *aeruginosa* in the Klamath River and San Francisco Bay delta, California USA.
753 *Aquatic Microbial Ecology* 57, 19–31. <https://doi.org/10.3354/ame01320>.
- 754 Munoz, G., Vo Duy, S., Roy-Lachapelle, A., Husk, B., Sauv e, S., 2017. Analysis of
755 individual and total microcystins in surface water by on-line preconcentration and
756 desalting coupled to liquid chromatography tandem mass spectrometry. *Journal of*
757 *Chromatography A* 1516, 9-20. <https://doi.org/10.1016/j.chroma.2017.07.096>.
- 758 Oehrle S.A., Southwell B., Westrick J., 2010. Detection of various freshwater
759 cyanobacterial toxins using ultra-performance liquid chromatography tandem mass
760 spectrometry. *Toxicon*. <https://doi.org/10.1016/j.toxicon.2009.10.001>.
- 761 Ogawa T., Misumi M., Sonoike K., 2017. Estimation of photosynthesis in cyanobacteria
762 by pulse-amplitude modulation chlorophyll fluorescence: problems and solutions.
763 *Photosynthesis Research* 133, 63–73. <https://doi.org/10.1007/s11120-017-0367-x>.
- 764 Orr P.T., Jones G.J., Douglas G.B., 2004. Response of cultured *Microcystis*
765 *aeruginosa* from the Swan River, Australia, to elevated salt concentration and
766 consequences for bloom and toxin management in estuaries. *Marine and*
767 *Freshwater Research* 55, 277. <https://doi.org/10.1071/MF03164>.
- 768 Ortiz X., Korenkova E., Jobst K.J., MacPherson K.A., Reiner E.J., 2017. A high
769 throughput targeted and non-targeted method for the analysis of microcystins and
770 anatoxin-A using on-line solid phase extraction coupled to liquid chromatography--
771 quadrupole time-of-flight high resolution mass spectrometry. *Analytical and*

- 772 Bioanalytical Chemistry 409, 4959–4969. [https://doi.org/10.1007/s00216-017-](https://doi.org/10.1007/s00216-017-0437-0)
773 0437-0.
- 774 Paerl H.W., Otten T.G, Kudela R., 2018. Mitigating the Expansion of Harmful Algal
775 Blooms Across the Freshwater-to-Marine Continuum. *Environmental Science &*
776 *Technology* 52, 5519–5529. <https://doi.org/10.1021/acs.est.7b05950>.
- 777 Paerl H.W., Paul V.J., 2012. Climate change: Links to global expansion of harmful
778 cyanobacteria. *Water Research* 46, 1349–1363.
779 <https://doi.org/10.1016/j.watres.2011.08.002>.
- 780 Paldavičiene A., Zaiko A., Mazur-Marzec H., Razinkovas-Baziukas A., 2015.
781 Bioaccumulation of microcystins in invasive bivalves: A case study from the boreal
782 lagoon ecosystem. *Oceanologia* 57, 93–101.
783 <https://doi.org/10.1016/j.oceano.2014.10.001>.
- 784 Peacock M.B., Gible C.M., Senn D.B., Cloern J.E., Kudela R.M., 2018. Blurred lines:
785 Multiple freshwater and marine algal toxins at the land-sea interface of San
786 Francisco Bay, California. *Harmful Algae* 73, 138–147.
787 <https://doi.org/10.1016/j.hal.2018.02.005>.
- 788 Preece E.P., Hardy F.J., Moore B.C., Bryan M., 2017. A review of microcystin
789 detections in Estuarine and Marine waters: Environmental implications and human
790 health risk. *Harmful Algae* 61, 31–45. <https://doi.org/10.1016/j.hal.2016.11.006>.
- 791 Reignier O, Bormans M, Lemarchand L, Siquin C, Amzil Z, Zykwincka A, and Briand
792 E. Production and composition of Extracellular Polymeric Substances (EPS) by a
793 unicellular strain and natural colonies of *Microcystis*: impact of salinity and nutrient
794 stress. *Environmental Microbiology Reports* 1-14. [https://doi.org/10.1111/1758-](https://doi.org/10.1111/1758-2229.13200)
795 2229.13200.
- 796 Réveillon, D., Abadie, E., Sechet, V., Briant, L., Savar, V., Bardouil, M., Hess, P., Amzil,
797 Z., 2014. Beta-N-Methylamino-L-Alanine: LC-MS/MS Optimization, Screening of
798 Cyanobacterial Strains and Occurrence in Shellfish from Thau, a French
799 Mediterranean Lagoon. *Marine Drugs* 12, 5441-5467.
800 <https://doi.org/10.3390/md12115441>.
- 801 Rippka R., Deruelles J., Waterbury J.B., Herdman M., Stanier R.Y., 1979. Generic
802 assignments, strain histories and properties of pure cultures of cyanobacteria.
803 *Microbiology* 111, 1–61.
- 804 Robson B.J., Hamilton D.P., 2003. Summer flow event induces a cyanobacterial bloom
805 in a seasonal Western Australian estuary. *Marine and Freshwater Research* 54,
806 139. <https://doi.org/10.1071/MF02090>.
- 807 Romera-García E., Helmus R., Ballesteros-Gómez A., Visser P.M., 2021. Multi-class
808 determination of intracellular and extracellular cyanotoxins in freshwater samples
809 by ultra-high performance liquid chromatography coupled to high resolution mass
810 spectrometry. *Chemosphere* 274, 129770.
811 <https://doi.org/10.1016/j.chemosphere.2021.129770>.
- 812 Ross C., Warhurst B.C., Brown A., Huff C., Ochrietor J.D., 2019. Mesohaline conditions
813 represent the threshold for oxidative stress, cell death and toxin release in the
814 cyanobacterium *Microcystis aeruginosa*. *Aquatic Toxicology* 206, 203–211.
815 <https://doi.org/10.1016/j.aquatox.2018.11.019>.

- 816
817 Svirčev Z., Lalić D., Bojadžija Savić G., Tokodi N., Drobac Backović D., Chen L.,
818 Meriluoto J., Codd G.A., 2019. Global geographical and historical overview of
819 cyanotoxins distribution and cyanobacterial poisonings. Springer Berlin Heidelberg.
820 <https://doi.org/10.1007/s00204-019-02524-4>.
- 821 Tatters, A.O., Howard, M.D.A., Nagoda, C., Busse, L., Gellene, A.G., Caron, D.A.,
822 2017. Multiple Stressors at the Land-Sea Interface: Cyanotoxins at the Land-Sea
823 Interface in the Southern California Bight. *Toxins* 9, 95.
824 <https://doi.org/10.3390/toxins9030095>.
- 825 Tatters, A.O., Smith, J., Kudela, R.M., Hayashi, K., Howard, M.D.A., Donovan, A.R.,
826 Loftin, K.A., Caron, D.A., 2021. The tide turns: Episodic and localized cross-
827 contamination of a California coastline with cyanotoxins. *Harmful algae* 103,
828 <https://doi.org/10.1016/j.hal.2021.102003>.
- 829 Teikari J.E., Hou S., Wahlsten M., Hess W.R., Sivonen L., 2018. Comparative
830 genomics of the Baltic Sea toxic cyanobacteria *Nodularia spumigena* UHCC 0039
831 and its response to varying salinity. *Frontiers in Microbiology* 9, 1–13.
832 <https://doi.org/10.3389/fmicb.2018.00356>.
- 833 Tonk L., Bosch K., Visser P., Huisman J., 2007. Salt tolerance of the harmful
834 cyanobacterium *Microcystis aeruginosa*. *Aquatic Microbial Ecology* 46, 117–123.
835 <https://doi.org/10.3354/ame046117>.
- 836 Tran, N.H., Li, Y.W., Reinhard, M., He, Y.L., Gin, K.Y.H., 2020. A sensitive and
837 accurate method for simultaneous analysis of algal toxins in freshwater using
838 UPLC-MS/MS and N-15-microcystins as isotopically labelled internal standards.
839 *Sci Total Environ* 738. <https://doi.org/10.1016/j.scitotenv.2020.139727>.
- 840 Turner A.D., Waack J., Lewis A., Edwards C., Lawton L., 2018. Development and
841 single-laboratory validation of a UHPLC-MS/MS method for quantitation of
842 microcystins and nodularin in natural water, cyanobacteria, shellfish and algal
843 supplement tablet powders. *Journal of Chromatography B* 1074–1075, 111–123.
844 <https://doi.org/10.1016/j.jchromb.2017.12.032>.
- 845 Vareli, K., Jaeger, W., Touka, A., Frillingos, S., Briasoulis, E., Sainis, I., 2013.
846 Hepatotoxic Seafood Poisoning (HSP) Due to Microcystins: A Threat from the
847 Ocean? *Marine Drugs* 11, 2751–2768. <https://doi.org/10.3390/md11082751>
- 848 Visser P.M., Verspagen J.M.H., Sandrini G., Stal L.J., Matthijs H.C.P, Davis T.W, Paerl
849 H.W., Huisman J., 2016. How rising CO₂ and global warming may stimulate harmful
850 cyanobacterial blooms. *Harmful Algae* 54, 145–159.
851 <https://doi.org/10.1016/j.hal.2015.12.006>.
- 852 Xiao M., Li M., Duan P., Qu Z., Wu H., 2019. Insights into the relationship between
853 colony formation and extracellular polymeric substances (EPS) composition of the
854 cyanobacterium *Microcystis* spp.. *Harmful Algae* 83, 34–41.
855 <https://doi.org/10.1016/j.hal.2019.02.006>.
- 856 Yoshizawa S., Matsushima R., Watanabe M.F., Harada K., Ichihara A., Carmichael
857 W.W., Fujiki H., 1990. Inhibition of protein phosphatases by *Microcystis* and
858 nodularin associated with hepatotoxicity. *Journal of Cancer Research and Clinical
859 Oncology* 116, 609–614. <https://doi.org/10.1007/BF01637082>.

860 Zendong, Z., McCarron, P., Herrenknecht, C., Sibat, M., Amzil, Z., Cole, R.B., Hess,
861 P., 2015. High resolution mass spectrometry for quantitative analysis and
862 untargeted screening of algal toxins in mussels and passive samplers. *Journal of*
863 *Chromatography A* 1416, 10-21. <https://doi.org/10.1016/j.chroma.2015.08.064>.

Journal Pre-proof

Acknowledgements: This research was funded by IFREMER and the Regional Council of the region des Pays de la Loire.

Conflicts of Interest: The authors declare no conflict of interest.

Journal Pre-proof

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Journal Pre-proof