

1 **First Detection of Paralytic Shellfish Poisoning (PSP) Toxins in**  
2 **Icelandic Mussels (*Mytilus edulis*): Links to Causative Phytoplankton**  
3 **Species.**

4

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16

17 **Abstract**

18 Paralytic shellfish poisoning (PSP) toxins were detected in blue mussels (*Mytilus*  
19 *edulis*) from two harvesting areas, Eyjafjordur on the north coast and Breidafjordur on  
20 the west coast of Iceland in 2009. During a bloom of *Alexandrium spp.* at both  
21 locations in June of that year, blue mussels were found to be contaminated with  
22 paralytic shellfish toxins (PSTs), leading to extensive closures of these harvesting  
23 sites.

24 Phytoplankton data taken during this time showed the presence of large numbers of *A.*  
25 *tamarensis*, with smaller numbers of *A. ostenfeldii* also being detected. Mussel

26 samples were analysed by mouse bioassay (MBA) and liquid chromatography with  
27 fluorescence detection (LC-FLD). Toxicity over 10 times the European Union (EU)  
28 regulatory limit was observed in samples from Eyjafjordur while levels over 4 times  
29 this limit were detected in samples from Breidafjordur. The toxin profile determined  
30 by LC-FLD was found to be composed primarily of the carbamate toxins  
31 gonyautoxin-2,3 (GTX-2,3). Saxitoxin (STX) was also detected in all samples  
32 analysed and was the second most abundant toxin present. Gonyautoxin-1,4 (GTX-  
33 1,4) was detected at lower concentrations in half the samples analysed from both  
34 locations. Comparison is made between predicted toxin profiles from these algal  
35 species and the toxin profiles determined through LC-FLD analysis.  
36 These results represent the first identification and PST profile determination in  
37 shellfish harvested from Icelandic waters.

38

### 39 **Keywords**

40 Paralytic shellfish poisoning (PSP); *Alexandrium*; Iceland; Liquid chromatography  
41 (LC); Mouse bioassay (MBA).

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### 44 **1. Introduction**

45 Paralytic shellfish poisoning (PSP) is caused by a group of 58 closely related  
46 compounds (Wiese *et al.*, 2010) based on a tetrahydropurine skeleton (Figure 1).

47 These toxins are mainly produced by marine dinoflagellates, in particular,

48 *Alexandrium* spp., *Gymnodinium catenatum* and *Pyrodinium bahamense* (EFSA

49 scientific opinion, 2009) but have also been found to be produced by freshwater

50 cyanobacteria (Onodera *et al.*, 1997; Dell'Aversano *et al.*, 2004). Shellfish feeding on

51 these algal species can accumulate the toxins without exhibiting adverse effects  
52 themselves.

53 Shellfish contaminated with these toxins pose severe risks to human consumers and  
54 numerous accounts of intoxications leading to illness or death have been recorded  
55 from around the world (IPCS 1984, Shumway et al., 1990, Gessner et al., 1997,  
56 Llewellyn et al., 2002, Garcia et al., 2004). The paralytic shellfish toxins (PSTs) act  
57 on mammalian cells by blocking the voltage-gated sodium channels (Catterall et al.,  
58 2007) leading to symptoms including, tingling sensation of the lips, mouth and  
59 tongue, numbness of the extremities, headache, dizziness, nausea, vomiting, diarrhoea  
60 and in severe cases death by asphyxiation (FAO/IOC/WHO, 2004).

61 The marine sector is hugely important to the Icelandic economy. In 2009 marine  
62 products accounted for 42% of Iceland's total export value with the industry  
63 employing approximately 7300 people, this represents nearly 4% of the overall  
64 workforce (Iceland Seafood Market Report, June 2010).

65 Shellfish have been harvested commercially in Iceland over the last 40 years with  
66 Icelandic scallop (*Clamys islandica*) and ocean quahog (*Artica islandica*) being the  
67 main species harvested. Mussel farming is relatively new however, with  
68 investigations into its feasibility being carried out in 1973 and 1985-87 (Icelandic  
69 Fisheries, 2011). Since these initial investigations blue mussels (*Mytilus edulis*) have  
70 been grown experimentally around the coast of Iceland with approximately 12 tonnes  
71 harvested in 2009, 32 tonnes in 2010 at 2 different harvesting locations and 94 tonnes  
72 in 2011 from approximately 6 different harvesting locations. A projected harvest  
73 figure for blue mussels of 100-120 tonnes is forecast in 2012. With nearly 5000 km of  
74 coastline, the Icelandic aquaculture industry has huge growth potential, making the

75 implementation of an effective biotoxin monitoring program a necessity if the  
76 European and world shellfish markets are to be tapped.

77 Harmful algal blooms (HABs) are a variable yet worldwide phenomenon and can  
78 pose severe economic risks especially to fledgling shellfish markets such as Iceland's.  
79 For human protection and as a statutory requirement, Iceland is obliged to conduct  
80 routine analysis of shellfish for regulated shellfish toxins from these harvesting sites.  
81 Community Regulation 853/2004 lays down specific hygiene rules for food of animal  
82 origin and stipulates the maximum permissible levels of PSTs in shellfish must not  
83 exceed 800 micrograms per kilogram before being placed on the market (Anon,  
84 2004).

85 Currently the mouse bioassay (MBA) is the reference method in Europe for PSP  
86 testing and involves extraction of shellfish homogenates with hydrochloric acid. As of  
87 the 6<sup>th</sup> November 2006 however, an LC-FLD method, also known as the "Lawrence  
88 Method", was written into EU legislation as an official alternative to the MBA (Anon,  
89 2006). Consequently a viable alternative exists in the legislation for those member  
90 states wishing to reduce or eliminate animal testing within the EU or for other third  
91 countries targeting EU export of bivalve molluscs or other shellfish products.

92 Since 2005, toxic species of phytoplankton have been monitored in three fjords  
93 around the coast of Iceland, Eyjafjordur on the central north coast, Breidafjordur on  
94 the northwest coast and Hvalfjordur on the southwest coast (Gudfinnsson et al., 2010).  
95 Phytoplankton is sampled weekly from spring to autumn and closure of these sites for  
96 harvesting shellfish is recommended when cell numbers exceed 500 cells/L of  
97 *Alexandrium* spp. (Gudfinnsson et al., 2010).

98 In this report we present data from the analysis of whole flesh mussel (*Mytilus edulis*)  
99 samples collected from two of these fjords located on the west and north coasts of

100 Iceland during a bloom of *Alexandrium* spp. in 2009. Samples were analysed for PSP  
101 toxicity by MBA with additional confirmatory analysis carried out by LC-FLD to  
102 determine toxin profiles and total saxitoxin equivalents. The contamination of blue  
103 mussels with PSTs in Iceland in 2009 represents a new and unique geographical  
104 location for the occurrence of these toxins, and one which may potentially result in a  
105 serious impact upon the livelihood of Icelandic shellfish producers and exporters.

106

## 107 **2. Methods and Materials**

108

### 109 **2.1 Chemicals**

110 All chemicals and solvents used were of analytical or HPLC grade. The water was  
111 supplied from a reverse osmosis system (Barnstead Int., Dubuque, IA, USA). Acetic  
112 acid, hydrochloric acid, ammonium formate, ammonium acetate, sodium chloride,  
113 sodium hydroxide, hydrogen peroxide, disodium hydrogen phosphate and periodic  
114 acid were purchased from Sigma-Aldrich (Steinheim, Germany). Acetonitrile was  
115 purchased from Labscan (Stillorgan, Ireland). Certified reference toxins: gonyautoxin  
116 1 and 4 (GTX-1,4), neosaxitoxin (NEO), decarbamoylsaxitoxin (dcSTX),  
117 gonyautoxin 2 and 3 (GTX-2,3), gonyautoxin 5 (GTX-5), N-sulfocarbamoyl-  
118 gonyautoxin 2 and 3 (C-1,2), decarbamoylneosaxitoxin (dcNEO),  
119 decarbamoylgonyautoxin 2 and 3 (dcGTX-2,3) and saxitoxin (STX) were obtained  
120 from the Institute of Marine Biosciences, National Research Council Canada (IMB,  
121 NRCC, Halifax, Nova Scotia, Canada). The certified reference materials (CRMs)  
122 were first diluted in water (adjusted to pH  $4 \pm 0.1$  with 0.1M acetic acid) to prepare  
123 primary stock solutions. Further dilutions were performed in 0.1mM acetic acid to

124 prepare working calibration solutions. Primary and working standards were stored  
125 following NRCC recommendations (Quilliam, 2007).

126

## 127 **2.2 Sample Material and Analysis:**

128

### 129 *2.2.1 Phytoplankton samples*

130 Samples were taken from two sites in Iceland: Eyjafjordur on the north coast and  
131 Breidafjordur on the west coast (Figure 2). There were two sampling sites in  
132 Breidafjordur: Flatey in the north of the fjord and Stykkisholmur in the south and one  
133 location in Eyjafjordur, Hrisey Island, located in the middle of the fjord.

134 Phytoplankton sampling was carried out weekly from spring to autumn. Toxic species  
135 were screened by net sampling using a 20 µm mesh. The net was hauled from a depth  
136 of 5 metres to the surface several times. All samples were fixed in hexamine buffered  
137 formalin and examined under a microscope. If toxic species were detected in these net  
138 samples then 50 ml water samples were allowed to settle in a sediment chamber for 24  
139 hours according to the Utermöhl method (Hasle, 1978) and examined in an inverted  
140 microscope where toxic species were identified and counted (Gudfinnsson et al.,  
141 2010).

142

### 143 *2.2.2 Mussel samples*

144 Samples were collected from two sites: Eyjafjordur (Hrisey Island) on the north coast  
145 and Breidafjordur (Stykkisholmur) on the west coast between June and August 2009.  
146 Mussels at both harvesting locations are grown in mesh sleeves attached to suspended  
147 long lines. Samples were stored in their shells at <-20°C until frozen samples were  
148 dispatched in one batch to the Marine Institute Ireland on ice.

149 The samples were thawed and prepared by dissecting and removing the whole flesh  
150 from the shell, removing byssus threads and any fragments of shell before being  
151 homogenised using a Waring blender (Hartford, CT, USA). The homogenised tissues  
152 were then extracted and analysed.

153

#### 154 *2.2.3 MBA analysis*

155 The MBA analysis involved acidic aqueous extraction in 0.1M HCl. Aliquots (1ml)  
156 were injected intraperitoneally into male albino CD1 strain mice in triplicate and  
157 toxicity ( $\mu\text{gSTXdiHCl-eq/kg}$ ) was calculated from median death times using  
158 Sommer's tables. The method was standardised using a certified reference standard of  
159 STX obtained from the Institute of Marine Biosciences, National Research Council  
160 Canada (IMB, NRCC, Halifax, Nova Scotia, Canada). The MBA procedure was  
161 carried out following AOAC official method 959.08.

162

#### 163 *2.2.4 Solid phase extraction (SPE)*

164 Sample cleanup was performed using Supelclean (Supelcosil, Bellefonte, PA, USA)  
165 C-18 cartridges (500 mg/3 ml). Ion exchange cleanup was performed to fractionate  
166 the C18-cleaned extracts using Bakerbond (J.T. Baker, Phillipsburg, NJ, USA) COOH  
167 cartridges (500 mg/3 ml) and both SPE steps followed the method specified in AOAC  
168 2005.06 (Anon, 2005b).

169

#### 170 *2.2.5 LC-FLD analysis*

171 The extraction, oxidation and analysis steps were carried out closely following the  
172 official method AOAC 2005.06. A Shimadzu (Kyoto, Japan) HPLC system with a  
173 fluorescence (FLD) detector (ex 340 nm, em 395 nm) (Shimadzu RF-10AXL) and

174 cooled autosampler (Shimadzu SIL-20A) was used. The HPLC column was a reverse  
175 phase C-18 Supelcosil (150 mm x 4.6 mm, 5 $\mu$ m) fitted with a C-18 Supelguard  
176 cartridge (20 mm). The HPLC programme followed was a slightly modified gradient  
177 elution based on that published in AOAC 2005.06 using a flow rate of 1.5 ml/min.  
178 The gradient followed was 0 – 5% mobile phase B over 5 min, 5 – 70 % B over the  
179 next 4 min, back to 0% B over 2 min, then keeping at this condition for 7 min before  
180 the next injection. PST concentrations in sample extracts were quantified against a  
181 five-point calibration for each toxin and are expressed in  $\mu$ mol/kg. Total saxitoxin  
182 equivalents were calculated for each sample as an estimation of total toxicity using the  
183 guidance described by the NRCC (Quilliam, 2007). The toxins GTX-2,3 and STX  
184 were quantitatively determined through direct analysis of SPE-C18 extracts and  
185 peroxide oxidation while the toxins GTX-1,4 were determined after ion-exchange  
186 SPE and periodate oxidation.

187

188

189

### 190 **3. Results**

191

#### 192 **3.1 Toxic Phytoplankton Species**

193 Results obtained from the Icelandic phytoplankton monitoring program have shown  
194 variable levels of toxic species present since 2005.

195

##### 196 *3.1.1 Breidafjordur*

197 Between the years 2005-2007, in Breidafjordur (Flatey), no *Alexandrium* spp. were  
198 found in any samples taken. In 2008 cell numbers exceeded 500 cells/L only once in



199 late May of that year (Gudfinnsson et al., 2010) but in June and July 2009 however,  
200 cell numbers of over 3500 cells/L were recorded at this site (data not shown).

201 *Alexandrium* spp. from the other sampling location in Breidafjordur (Stykkisholmur)  
202 have been found infrequently and in very low numbers in the years 2005-2008. In  
203 2009 however high densities of cells were found, starting in late June and persisting  
204 until the middle of July, peaking at over 16000 cells/L (Table 1).

205

### 206 3.1.2 Eyjafjordur

207 At Hrisey Island in Eyjafjordur, *Alexandrium* spp. have been observed each year from  
208 2005 – 2008 with cell densities > 6000 cells/L found in 2005 (data not shown). In  
209 2009 *Alexandrium* spp. peaked twice, firstly at over 8,000 cells/L in June and  
210 secondly at over 10,000 cells/L in July (Table 1).

211 The *Alexandrium* populations detected in phytoplankton samples from both fjords  
212 were mainly composed of *A. tamarense* with small numbers of *A. ostenfeldii* being  
213 found in the highly concentrated samples taken.

214

## 215 3.2 MBA and LC-FLD toxicity data

216

### 217 3.2.1 Breidafjordur

218 The MBA and LC-FLD toxicity data from Breidafjordur is presented in Table 2. The  
219 first mussel sample was collected on the 30/06/09 when toxicity was already over  
220 three times the regulatory limit. The toxicity rose to over 4 times this limit by the  
221 second sample taken on the 10/07/09 before dropping over the next 4 weeks to levels  
222 below this regulatory action level. The highest total toxicity result was observed in  
223 sample 2, with an MBA result of over 4500  $\mu\text{gSTXdiHCl-eq/kg}$ .

224

225 *3.2.2 Eyjafjordur*

226 The MBA and LC-FLD toxicity data generated from the analysis of the Eyjafjordur  
227 mussel samples collected during 2009 is presented in Table 3. Toxicity was found to  
228 be below but close to the regulatory action limit of 800  $\mu\text{gSTXdiHCl-eq/kg}$  in early  
229 June, seen in sample 1, but was found to rise quickly to nearly 10 times the limit  
230 within the subsequent two weeks, sample 2. This re-emphasises the speed with which  
231 these toxins can accumulate in shellfish tissue. Toxicity levels remained high for a  
232 further 6-8 weeks and did not drop to within regulatory limits until the end of August,  
233 sample 5. The highest total toxicity result was observed in sample 3, with results by  
234 LC-FLD and MBA of over 8500  $\mu\text{gSTXdiHCl-eq/kg}$ .

235

236 Chromatograms taken after peroxide and periodate oxidation of a sample from  
237 Breidafjordur are presented in Figures 3 and 4. Results from both fjords showed the  
238 absence of any other toxin oxidation product peaks which may relate to other PSP  
239 toxins or metabolic products. Analysis of unoxidised extracts of the samples revealed  
240 no interfering matrix co-extractives (data not shown) which may have interfered with  
241 the qualitative identification of the PSP toxins and subsequently compromised toxin  
242 quantitation.

243

244 **4. Discussion**

245 Conditions within both fjords during the sampling periods were favourable for  
246 phytoplankton growth as confirmed through the data presented in Table 1, where cell  
247 counts of *Alexandrium* spp. reached record levels in both Eyjafjordur and  
248 Breidafjordur. The exact causes of the high cell numbers observed is unknown and

249 could be due to a number of factors. Temperature and salinity increases along the west  
250 and north coasts have been observed over the last decade due to a stronger inflow of  
251 Atlantic waters into these grounds (Gudfinnsson et al, 2010). It is unclear from results  
252 obtained to date whether these trends are related in any way to the effects of climate  
253 change or, as is more probable, relate to natural cyclic variations such as oscillations  
254 to the North Atlantic subpolar gyre (Hátún et al, 2005, Hátún et al, 2009). Warmer  
255 more saline subtropical waters can spread north and westwards when this gyre  
256 weakens, as it controls the flow trajectory of the North Atlantic Current. A weakening  
257 of this gyre has been observed over the last decade which could explain the  
258 temperature and salinity increases observed by Gudfinnsson et al (2010).

259 A comparison of the results obtained from both the algal cell counts and the toxicity  
260 tests are illustrated in Figure 5. A clear correlation is evident between the high cell  
261 counts recorded and flesh samples containing higher concentrations of PSTs. Notably,  
262 the data from Breidafjordur suggests a level of time delay between the highest  
263 concentrations of algae and toxin levels recorded in the flesh. There is also a clear  
264 relationship between the reduction of algal cells and the total toxicity determined in  
265 the flesh samples. Unfortunately, an absence of flesh samples collected from  
266 Eyjafjordur in July 2009 prevents an actual comparison between the toxicity of the  
267 flesh and the Alexandrium cell count during the second algal bloom at this location.

268

#### 269 **4.1 Toxin Profile Determination**

270 The LC-FLD method has been proven as a valuable tool in the qualitative and  
271 quantitative determination of PSP toxins in shellfish (Turner et al., 2009). The  
272 epimeric pairs (e.g. GTX-2 and GTX-3, GTX-1 and GTX-4, C-1 and C-2 and dcGTX-  
273 2 and dcGTX-3) are not separated analytically using this LC-FLD method (AOAC

274 2005.06) and are therefore presented as a combined sum using the higher toxicity  
275 factor of the two co-eluted epimers to calculate total toxicity. Through analysis using  
276 this method the toxin profile was determined and found to be similar in both fjords  
277 with samples predominated by the carbamate toxins GTX-2,3. STX was the next most  
278 abundant toxin present with GTX-1,4 observed in half the samples analysed (Figure  
279 6).

280 The toxin profiles determined in these samples are similar to those found in other  
281 areas where *Alexandrium* spp. predominates such as the UK (Turrel et al., 2007)  
282 where the toxins GTX-2,3 and STX predominate with lower levels of GTX-1,4, NEO  
283 and GTX-5 also being found, or in Ireland where GTX-2,3 has been found to  
284 predominate with lower relative concentrations of STX and GTX-1,4 being  
285 determined (Furey et al., 1998, Marine Institute Ireland internal data). Interestingly,  
286 there is no indication of the presence of any of the N-sulfocarbamoyl toxins such as  
287 C-1,2, which have been found to occur in mussels containing PSP toxins in some UK  
288 waters since 2008 (Turner, personal communication) and which are associated with a  
289 number of different strains of *Alexandrium* spp. The Norwegian PSP toxin profile  
290 typically observed is slightly different to that observed in Iceland, being predominated  
291 by GTX-1,4, with both NEO and STX being found at lower relative concentrations  
292 (Sayfritz et al., 2008). The differences between profiles in the region and Iceland's is  
293 mainly the absence of the toxins NEO and C-1,2 from samples analysed.

294 Profiles of *A. tamarense* mainly consist of the N-sulfocarbamoyl toxins, C-1,2 and the  
295 high potency carbamate toxins GTX-1-4, NEO and STX (Ichimi et al., 2002, Persich  
296 et al., 2006). Profiles of *A. ostenfeldii* can contain the spirolides as well as the PSTs  
297 GTX-6, C-1,2 and GTX-2,3 (Hansen et al., 1992, Ciminiello et al., 2006). The  
298 absence of the N-sulfocarbamoyl toxins C-1,2 from mussel samples taken from both

299 harvesting areas, if not relating to the toxin profile in the source algae, could instead  
300 be due to the metabolic conversion of these toxins in shellfish to GTX-2,3 via  
301 desulfonation and epimerization (Krock et al., 2007).

302 This hypothesis could explain the high concentrations of GTX-2,3 found in samples  
303 as evidenced in Figure 6. The percentage toxin profile presented in this figure shows  
304 similarities between both fjords with GTX-2,3 being the predominant toxins present  
305 in early samples taken in June and early August, although a discrepancy is noted in  
306 the data set with STX being the predominant toxin found in the Eyjafjordur sample  
307 from the 08/06/09. The ratio of GTX-2,3 to STX changes by late August with STX  
308 becoming the predominant toxin present. Again this could relate either to changes in  
309 the toxin ratios present within the algal food source or alternatively relate to the  
310 potential toxin transformation of GTX-2,3 to STX via desulfonation (Fast et al.,  
311 2006). However it is noted that the in vitro experiments carried out by Fast et al.,  
312 were only carried out in clam tissues.

313 It is interesting to note that although the *Alexandrium* cell counts found in  
314 Breidafjordur (figure 5) were considerably higher than those found in Eyjafjordur, the  
315 same ratio was not evident in the toxicity results of the mussel samples. The total PSP  
316 toxicity found in mussels from Eyjafjordur was nearly twice that found in mussels  
317 from Breidafjordur.

318 The absence of GTX-1,4 in samples taken in early June and late August from  
319 Eyjafjordur and early August onwards from Breidafjordur is likely due to the low  
320 overall toxicity of these samples and the lower relative sensitivity of the N-  
321 hydroxylated toxins to their non-hydroxylated counterparts when analysed using  
322 method AOAC 2005.06 (Turner et al., 2009).

323 It is imperative therefore to have adequate knowledge of specific toxin profiles for the  
324 analysis and risk management of this group of potent neurotoxins due to the range of  
325 relative toxicities exhibited by the various analogues. These results highlight the  
326 presence in Iceland of some of the most toxic PSP toxins as well as levels of toxicity  
327 which may at times provide a serious risk to the human consumer.

328

#### 329 **4.2 Chemical and Biological Method Analysis**

330 Toxicity results returned by both the reference MBA method and the LC-FLD method  
331 appear to correlate reasonably well for these samples (Figure 5), as observed  
332 previously in this species for mussels sampled from within UK waters (Turner et al.,  
333 2009). Overall the MBA method gave slightly higher values compared to the LC-FLD  
334 as evidenced in tables 2 and 3, although a variability in this ratio is noted.

335 It is also clear from the results generated from samples 4-6 from Breidafjordur (Table  
336 2), that the LC-FLD method provides useful data on the toxicity of samples  
337 containing levels of PSTs lower than the MBA limit of quantitation. This again shows  
338 the usefulness of the LC-FLD method for the early warning of toxicity, especially  
339 important given the rapid increases in PSP toxin levels observed in these areas (Table  
340 3). These results therefore clearly demonstrate the importance of a regular effective  
341 toxicity monitoring regime, without which there would be a clear potential risk to  
342 human consumers to toxic bloom events.

343 The level of observed time delay between the peaks in phytoplankton cell presence  
344 found in the water and the maximum levels of toxicity found in shellfish (Figure 5) is  
345 also of interest. At Breidafjordur, the peak in toxicity appears approximately two  
346 weeks after the measured maximum of Alexandrium cells. This observation is  
347 consistent with those observed previously from water and flesh samples collected in

348 the St. Lawrence region, Canada (Blasco et al., 2003) or from Busta Voe Lee North,  
349 Scotland (CEFAS Contract Report C2649) where time delays of over 7 days have  
350 been found.

351

352

### 353 **5. Conclusions**

354 These findings represent a first report of these toxins in mussel samples from Iceland  
355 and furthermore indicate the potential increase in the presence of the toxins and  
356 causative phytoplankton over the past few years. It is difficult to ascertain however, if  
357 this increase is due to the application of phytoplankton monitoring in Icelandic waters  
358 or truly represents an increase in the incidence of these toxic dinoflagellates. With the  
359 increasing economic importance placed upon the shellfish industry in Iceland, this  
360 highlights the importance of continued monitoring of both shellfish toxicity and their  
361 causative organisms, in order to produce a full and thorough risk assessment for the  
362 occurrence of PSP in Icelandic waters so as to provide the necessary information to  
363 ensure an appropriate biotoxin monitoring programme is continued. Ongoing work  
364 will continue with the analysis of both water and flesh samples from both current and  
365 developing shellfish harvesting beds and over time build up more data on the timing  
366 and intensity of the algal blooms and the subsequent shellfish toxin accumulation.  
367 Further data will allow the ongoing assessment of the presence and variability of PSP  
368 toxicity and toxin profiles, ultimately providing an essential resource to ensure the  
369 continued development of the Icelandic shellfish production program.

370

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375 provided shellfish samples and phytoplankton data.

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524 **List of Figures (N.B. all figures intended for full colour reproduction on the web**  
525 **and in print)**

526

527 **Figure 1.** Structure of the PSP toxins.

528

529 **Figure 2.** Map showing the production areas on the north and west coasts of Iceland  
530 in 2009.

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532 **Figure 3.** LC-FLD separation of PSP toxins present in an Icelandic sample from  
533 Breidafjordur in 2009 after SPE-C18 cleanup and peroxide oxidation.

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535 **Figure 4.** LC-FLD separation of PSP toxins present in an Icelandic sample from  
536 Breidafjordur in 2009 after SPE-ion exchange cleanup and periodate oxidation.

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538 **Figure 5.** Comparison of Alexandrium Cell Counts in the water (cells/L) and total  
539 sample toxicity of the harvested mussels ( $\mu\text{g STX diHCl eq./kg}$ ) returned by both LC-  
540 FLD and MBA in Eyjafjordur and Breidafjordur.

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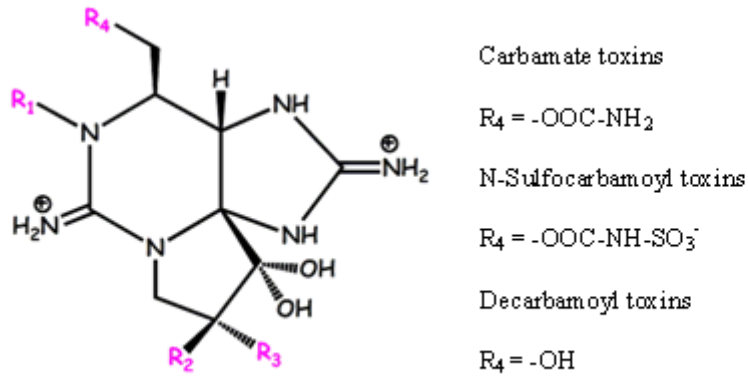
542 **Figure 6.** Percentage PST profiles determined by LC-FLD (in terms of  $\mu\text{mol/kg}$ ) from  
543 mussel samples collected from Eyjafjordur and Breidafjordur.

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Figure 1



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Figure 2



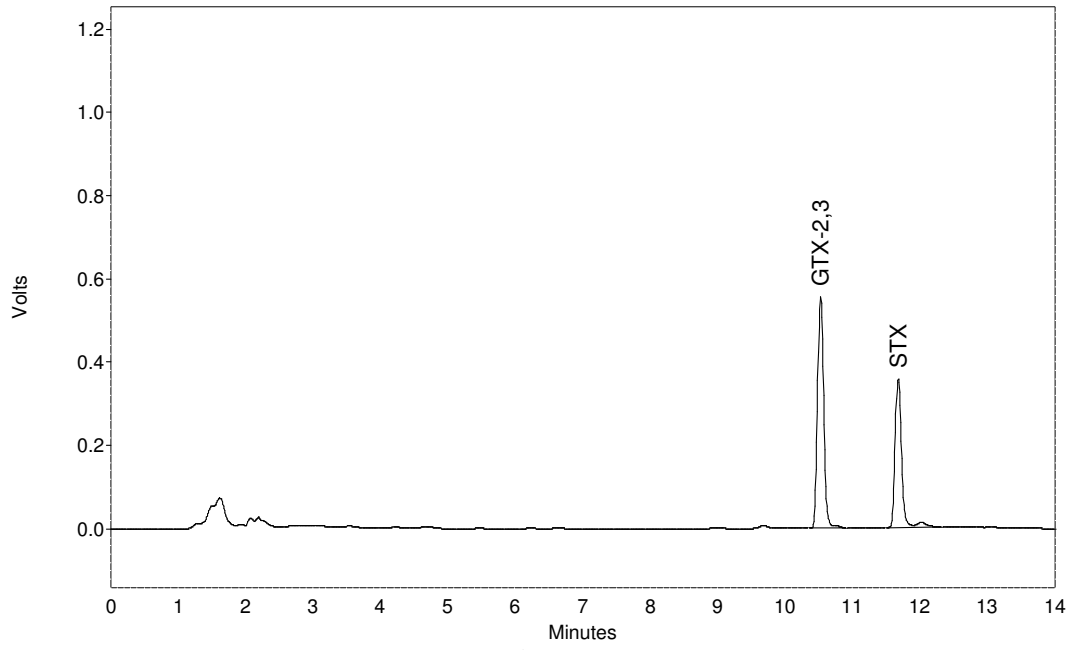


Figure 3

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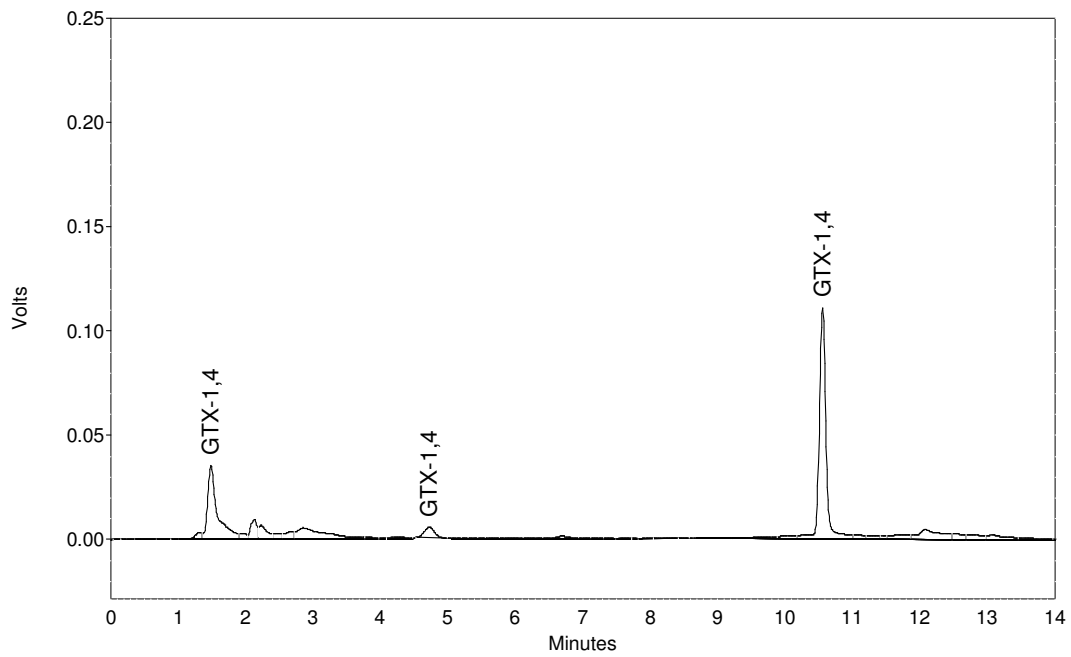


Figure 4

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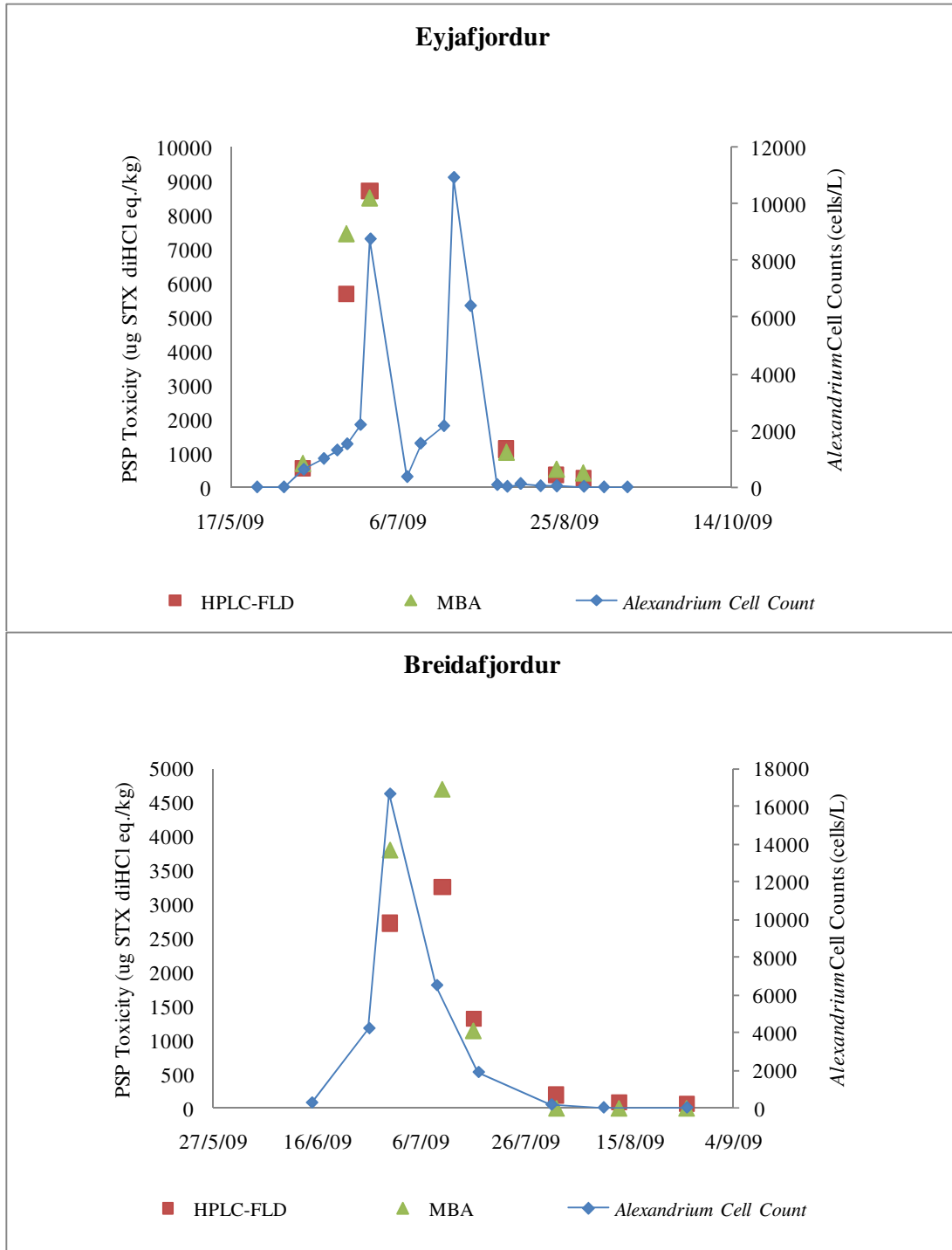


Figure 5

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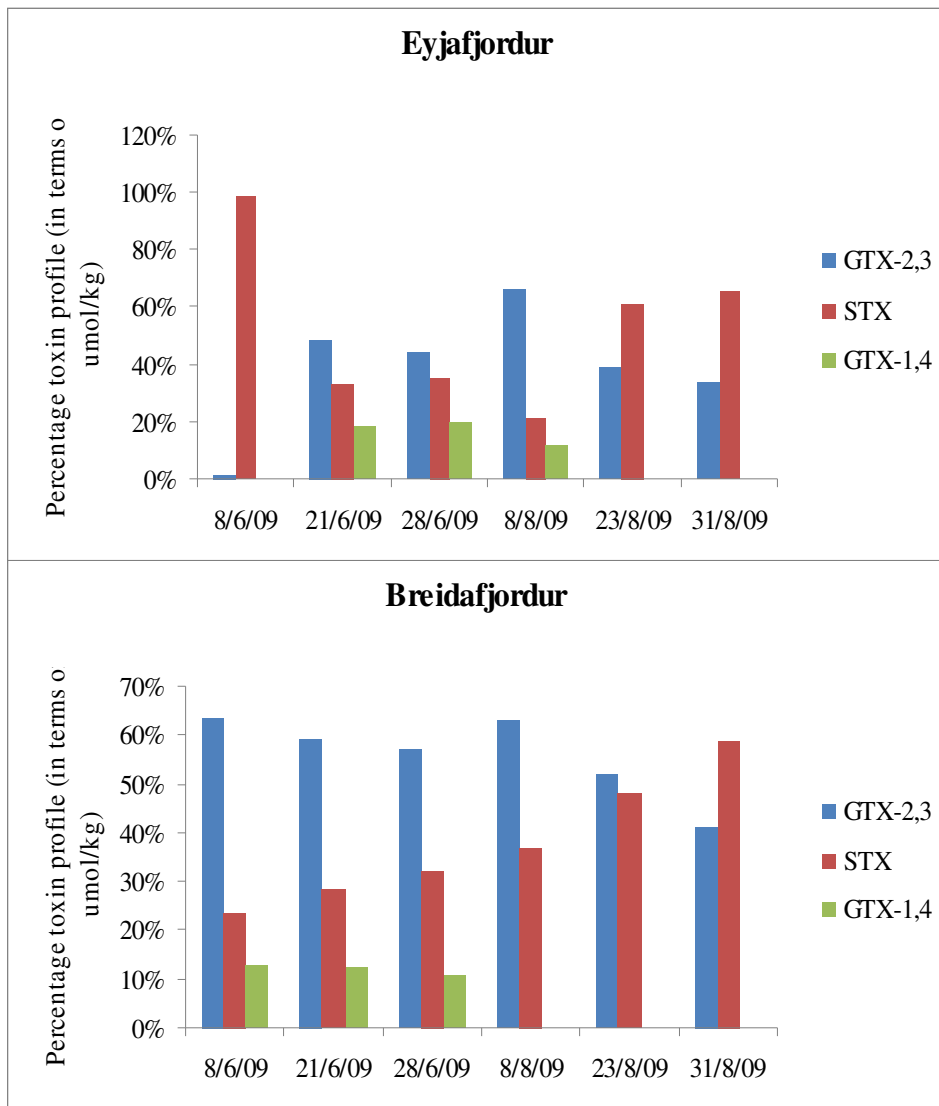


Figure 6

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598 **List of Tables**

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600 **Table 1:** Phytoplankton cell counts taken during May to September 2009 from  
601 Eyjafjordur and Breidafjordur (Stykkisholmur), Iceland.

602

603 **Table 2:** MBA and HPLC-FLD data of mussel (*M.edulis*) samples harvested from  
604 Breidafjordur, Iceland.

605

606 **Table 3:** MBA and HPLC-FLD data of mussel (*M.edulis*) samples harvested from  
607 Eyjafjordur, Iceland.

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648 **Table 1**

Sample	Sampling Date	Cell Counts (Alex spp.) cells/L	
		Eyjafjardur	Breidafjardur (Stykkisholmur)
1	25/05/2009	0	-
2	02/06/2009	0	-
3	08/06/2009	620	-
4	14/06/2009	1000	-
5	15/06/2009	-	260
6	18/06/2009	1300	-
7	21/06/2009	1520	-
8	25/06/2009	2200	-
9	26/06/2009	-	4208
10	28/06/2009	8750	-
11	30/06/2009	-	16680
12	09/07/2009	360	6500
13	13/07/2009	1540	-
14	17/07/2009	-	1880
15	20/07/2009	2160	-
16	23/07/2009	10920	-
17	28/07/2009	6400	-
18	31/07/2009	-	160
19	05/08/2009	80	-
20	08/08/2009	20	-
21	10/08/2009	-	0
22	12/08/2009	120	-
23	18/08/2009	40	-
24	23/08/2009	60	-
25	26/08/2009	-	0
26	31/08/2009	20	-
27	06/09/2009	0	-
28	13/09/2009	0	-

649 (-) no samples taken

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663 **Table 2**

Sample	Sampling Date	Concentration ( $\mu\text{mol/kg}$ )			Total Toxicity $\mu\text{gSTXdiHCl-eq./kg}$	
		GTX-2,3	STX	GTX-1,4	HPLC-FLD	MBA
1	30/06/2009	6.06	2.25	1.24	2733	3800
2	10/07/2009	6.60	3.18	1.39	3263	4694
3	16/07/2009	2.55	1.44	0.47	1318	1141
4	01/08/2009	0.41	0.24	n.d	186	<LOQ
5	13/08/2009	0.13	0.12	n.d	76	<LOQ
6	26/08/2009	0.07	0.10	n.d	56	<LOQ

664

n.d. Toxin not detected

665

Gonyautoxin (GTX), saxitoxin (STX)

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LOQ for MBA 280  $\mu\text{gSTXdiHCl-eq./kg}$ 

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705 **Table 3**

Sample	Sampling Date	Concentration ( $\mu\text{mol/kg}$ )			Total Toxicity $\mu\text{gSTXdiHCl-eq./kg}$	
		GTX-2,3	STX	GTX-1,4	HPLC-FLD	MBA
1	08/06/2009	0.02	1.44	n.d	540	720
2	21/06/2009	9.05	6.18	3.39	5700	7460
3	28/06/2009	12.40	9.97	5.60	8728	8510
4	08/08/2009	2.63	0.86	0.48	1123	1050
5	23/08/2009	0.45	0.70	n.d	368	550
6	31/08/2009	0.28	0.54	n.d	266	440

706

n.d. Toxin not detected

707

Gonyautoxin (GTX), saxitoxin (STX)

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