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

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Abstract

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

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

samples were analysed by mouse bioassay (MBA) and liquid chromatography with fluorescence detection (LC-FLD). Toxicity over 10 times the European Union (EU) regulatory limit was observed in samples from Eyjafjordur while levels over 4 times this limit were detected in samples from Breidafjordur. The toxin profile determined by LC-FLD was found to be composed primarily of the carbamate toxins gonyautoxin-2,3 (GTX-2,3). Saxitoxin (STX) was also detected in all samples analysed and was the second most abundant toxin present. Gonyautoxin-1,4 (GTX-1,4) was detected at lower concentrations in half the samples analysed from both locations. Comparison is made between predicted toxin profiles from these algal species and the toxin profiles determined through LC-FLD analysis.

These results represent the first identification and PST profile determination in shellfish harvested from Icelandic waters.

Keywords

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

1. Introduction

Paralytic shellfish poisoning (PSP) is caused by a group of 58 closely related compounds (Wiese *et al.,* 2010) based on a tetrahydropurine skeleton (Figure 1). These toxins are mainly produced by marine dinoflagellates, in particular, *Alexandrium* spp*., Gymnodinium catenatum* and *Pyrodinium bahamense* (EFSA scientific opinion, 2009) but have also been found to be produced by freshwater cyanobacteria (Onodera *et al.*, 1997; Dell'Aversano *et al.,* 2004). Shellfish feeding on

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

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

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

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

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

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

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

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

In this report we present data from the analysis of whole flesh mussel (*Mytilus edulis*) 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 ± 0.1 with 0.1M acetic acid) to prepare
123 primary stock solutions. Further dilutions were performed in 0.1mM acetic acid to

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

2.2 Sample Material and Analysis:

2.2.1 Phytoplankton samples

Samples were taken from two sites in Iceland: Eyjafjordur on the north coast and Breidafjordur on the west coast (Figure 2). There were two sampling sites in Breidafjordur: Flatey in the north of the fjord and Stykkisholmur in the south and one location in Eyjafjordur, Hrisey Island, located in the middle of the fjord. Phytoplankton sampling was carried out weekly from spring to autumn. Toxic species were screened by net sampling using a 20 µm mesh. The net was hauled from a depth of 5 metres to the surface several times. All samples were fixed in hexamine buffered formalin and examined under a microscope. If toxic species were detected in these net samples then 50 ml water samples were allowed to settle in a sediment chamber for 24 hours according to the Utermöhl method (Hasle, 1978) and examined in an inverted microscope where toxic species were identified and counted (Gudfinnsson et al., 2010).

2.2.2 Mussel samples

Samples were collected from two sites: Eyjafjordur (Hrisey Island) on the north coast and Breidafjordur (Stykkisholmur) on the west coast between June and August 2009. Mussels at both harvesting locations are grown in mesh sleeves attached to suspended long lines. Samples were stored in their shells at <-20^{o}C until frozen samples were 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 (µgSTXdiHCl-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

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

3. Results

3.1 Toxic Phytoplankton Species

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

3.1.1 Breidafjordur

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

late May of that year (Gudfinnsson et al., 2010) but in June and July 2009 however, cell numbers of over 3500 cells/L were recorded at this site (data not shown). *Alexandrium* spp. from the other sampling location in Breidafjordur (Stykkisholmur) have been found infrequently and in very low numbers in the years 2005-2008. In 2009 however high densities of cells were found, starting in late June and persisting until the middle of July, peaking at over 16000 cells/L (Table 1).

3.1.2 Eyjafjordur

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

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

3.2 MBA and LC-FLD toxicity data

3.2.1 Breidafjordur

The MBA and LC-FLD toxicity data from Breidafjordur is presented in Table 2. The first mussel sample was collected on the 30/06/09 when toxicity was already over three times the regulatory limit. The toxicity rose to over 4 times this limit by the second sample taken on the 10/07/09 before dropping over the next 4 weeks to levels below this regulatory action level. The highest total toxicity result was observed in sample 2, with an MBA result of over 4500 µgSTXdiHCl-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 µgSTXdiHCl-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 µgSTXdiHCl-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

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

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

4.1 Toxin Profile Determination

The LC-FLD method has been proven as a valuable tool in the qualitative and quantitative determination of PSP toxins in shellfish (Turner et al., 2009). The epimeric pairs (e.g. GTX-2 and GTX-3, GTX-1 and GTX-4, C-1 and C-2 and dcGTX-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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379 **References**

380

381 Anon 2004, Commission Regulation (EC) No. 853/2004 of 29th April 2004 of the
382 European Parliament and of the Council of 29th April 2004 laying down specific
383 hygiene rules for food of animal origin. L226/22-80

384 Anon 2005a. AOAC Official Method 959.08. Paralytic Shellfish Poison. Biological
385 method. Final Action. In: AOAC Official Methods of Analysis, 18th Edition Chapter
386 49: Natural Toxins (chapter ed. M.W. Truckses), pp79-80. Gaithersburg, MD, USA:
387 AOAC International.

388 Anon 2005b. AOAC Official Method 2005.06 Quantitative determination of Paralytic
389 Shellfish Poisoning Toxins in shellfish using pre-chromatographic oxidation and
390 liquid chromatography with fluorescence detection, 18th Edition. Gaithersburg, MD,
391 USA: AOAC International.

392

393 Anon 2006, Commission Regulation (EC) No 1664/2006 of 6th Nov. 2006 amending
394 Regulation (EC) No 2074/2005 as regards implementing measures for certain
395 products of animal origin intended for human consumption and repealing certain
396 implementing measures. Official Journal of the European Union. L320, 13-45.

397

398 Blasco D., Levasseur M., Bonneau E., Gelinas R., and Packard T.T., 2003. Patterns of
399 paralytic shellfish toxicity in the St. Lawrence region in relationship with the
400 abundance and distribution of *Alexandrium tamarense*. Scientia Marina 67(3): 261-
401 278.

402

403 Catterall, W.A., Ceste` le, S., Yarov-Yarovoy, V.,Yu,F.H., Konoki,K., Scheuer, T.,
404 2007. Voltage-gated ion channels and gating modifier toxins. Toxicon 49,124–141.
405
406 CEFAS Contract Report – C2649. Biotoxin Monitoring Report for Scotland.
407 Monitoring for Paralytic Shellfish Poisoning toxins and lipophilic toxins - 1st April
408 2006 to 31st March 2007 Final Report on behalf of The Food Standards Agency
409 Scotland Contract Reference: PAU 179 – S02007/PSP & DSP.
410
411 Ciminiello P., Dell'Aversano C., Fattorusso E., Magno S., Tartaglione L., Cangini M.,
412 Pompei M., Guerrini F., Boni L., and Pistocchi R., 2006. Toxin profile of
413 *Alexandrium ostenfeldii* (Dinophyceae) from the Northern Adriatic Sea revealed by
414 liquid chromatography-mass spectrometry. Toxicon 47: 597-604
415
416 Dell'Aversano C., Eaglesham GK., and Quilliam M.A., 2004. Analysis of
417 cyanobacterial toxins by hydrophilic interaction liquid chromatography – mass
418 spectrometry. J Chrom A 1028 (1), 155-164
419
420 FAO/IOC/WHO (Food and Agriculture Organization of the United
421 Nations/Intergovernmental Oceanographic Commission of UNESCO/World Health
422 Organization), 2004. In Background document of the Joint FAO/IOC/WHO ad hoc
423 Expert Consultation on Biotoxins in Bivalve Molluscs, Oslo, Norway, September 26
424 30, 2004.
425

426 Fast M. D., Cembella A. D., Ross N.W., 2006. In vitro transformation of paralytic
427 shellfish toxins in the clams *Mya arenaria* and *Protothaca staminea*. Harmful Algae
428 5:79-90
429
430 Furey, A., James, K.J., Sherlock, I.R., 1998. First report of paralytic shellfish
431 poisoning toxins in the Republic of Ireland. In: Reguera, B., Blanco, J., Fernandez,
432 M.L., Wyatt, T. (Eds.), Harmful Algae. Xunta de Galicia and Intergovernmental
433 Oceanographic Commission of UNESCO, pp. 70–71.
434
435 Garcia C., del Carmen Bravo M., Lagos M. and Lagos N., 2004. Paralytic shellfish
436 poisoning: post-mortem analysis of tissue and body fluid samples from human victims
437 in the Patagonia fjords. *Toxicon* 43 (2), 149-158.
438
439 Gessner B.D., Bell P., Doucette G.J., Moczydlowski E., Poli M.A., Van Dolah F. and
440 Hall S., 1997. Hypertension and identification of toxin in human urine and serum
441 following a cluster of mussel-associated paralytic shellfish poisoning outbreaks.
442 *Toxicon* 35 (5), 711-722.
443
444 Gudfinnsson H. G., Eydal A., Gunnarsson K., Gudmundsson K., and Valsdóttir K.,
445 2010, Monitoring of toxic phytoplankton in three Icelandic fjords, ICES theme
446 session N, ICES CM 2010/N:12, p.6
447
448 Hansen P.J., Cembella A.D., Moestrup Ø., 1992, The marine dinoflagellate
449 *Alexandrium ostenfeldii*: paralytic shellfish toxin concentration, composition and
450 toxicity to a tintinnid ciliate. Journal of Phycology 28: 597-603

451 Hasle, G.R., 1978, Using the inverted microscope. In: Phytoplankton Manual.
452 Sournia, A. (ed), UNESCO, Paris: 191-196
453
454 Hátún, H., Sando, A.B., Drange, H., Hansen, B and Valdimarsson, H., 2005. Influence
455 of the Atlantic subpolar gyre on the thermohaline circulation. Science (Washington,
456 D.C.), 309: 1841-1844. Doi:10.1126/science. 1114777. PMID:16166513.
457
458 Hátún H., Payne M.R., and Jacobsen J.A., 2009, The North Atlantic subpolar gyre
459 regulates the spawning distribution of blue whiting (*Micromesistius poutassou*). Can.
460 J. Aquat. Sci. 66: 759-770
461
462 Iceland Seafood Market Report, June 2010, p.5, Islandsbanki, Reykjavik, Iceland.
463 [http://www.islandsbanki.is/servlet/file/store156/item64129/version2/Seafood%20rep
464 ort%202010%2005%20vef.pdf] accessed 21st January 2011.
465
466 Icelandic Fisheries, Information centre of the Icelandic Ministry of Fisheries and
467 Agriculture. [http://www.fisheries.is/aquaculture/species/blue-mussel/] accessed 21st
468 January 2011.
469
470 Ichimi K., Suzuki T., and Ito A., 2002. Variety of PSP toxin profiles in various culture
471 strains of *Alexandrium tamarense* and change of toxin profile in natural *A. tamarense*
472 population. Journal of Experimental Marine Biology and Ecology 273: 51-60.
473

474 IPCS (International Programme on Chemical Safety), 1984. Aquatic (Marine and
475 Freshwater) Biotoxins. In: Environmental Health Criteria 37. World Health
476 Organization, ISBN 92 4 154097 4.
477
478 Krock B., Seguel C. G., Cembella A. D., 2007. Toxin profile of *Alexandrium*
479 *catenella* from the Chilean coast as determined by liquid chromatography with
480 fluorescence detection and liquid chromatography coupled with tandem mass
481 spectrometry. Harmful Algae 6:734-744.
482
483 Llewellyn L., Dodd M.J., Robertson A., Ericson G., deKoning C., Negri A.P., 2002.
484 Post-mortem analysis of samples from a human victim of a fatal poisoning caused by
485 the xanthid crab, *Zosimus aeneus.* Toxicon 40, 1463-1469
486
487 Onodera H., Satake M., Oshima Y., Yasumoto T. and Carmichael W.W., 1997. New
488 Saxitoxin analogues from the freshwater filamentous cyanobacterium *Lyngbya wollei*.
489 *J Nat Tox 5:146-151*
490
491 Persich G.R., Kulis D.M., Lilly E.L., Anderson D.M., Garcia V.M.T., 2006, Probable
492 origin and toxin profile of *Alexandrium tamarense* (Lebour) Balech from southern
493 Brazil. Harmful Algae 5: 36-44.
494
495 M.A. Quilliam. “Supplemental Information for PSP Toxin CRMs: Structures,
496 Molecular Weights, Concentrations and Toxicities”, CRMP Technical Report CRM-
497 PSP-20070411, National Research Council Canada, Halifax, April 2007.
498

499 Sayfritz S.J., Aasen J.A.B., Aune T., 2008, Determination of paralytic shellfish
500 poisoning toxins in Norwegian shellfish by liquid chromatography with fluorescence
501 and tandem mass spectrometry detection. Toxicon 52:330-340
502
503 Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from
504 the European Commission on Marine Biotoxins in Shellfish – Saxitoxin Group. *The*
505 *EFSA Journal* (2009) 1019, 1-76
506
507 Shumway, S.E., 1990. A review of the effects of algal blooms on shellfish and
508 aquaculture. J. World Aquacul. Soc. 21, 65-104
509
510 Turner A.D., Norton D.M., Hatfield R.G., Morris S., Reese A.R., Algoet M. and Lees
511 D.N, 2009. Refinement and extension of AOAC method 2005.06 to include additional
512 toxins in mussels: Single-laboratory validation. J. AOAC International. 92 (1), 190-
513 207.
514
515 Turrel E.A., Lacaze J.P., Stobo L., 2007, Determination of paralytic shellfish
516 poisoning (PSP) toxins in UK shellfish. Harmful Algae 6:438-448
517
518 Wiese M., D'Agostino P. M., Mihali T. K., Moffitt M. C. and Neilan B. A., 2010,
519 Neurotoxic Alkaloids: Saxitoxin and its Analogues. Marine Drugs 8:2185-2211
520
521
522
523

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

Figure 1. Structure of the PSP toxins.

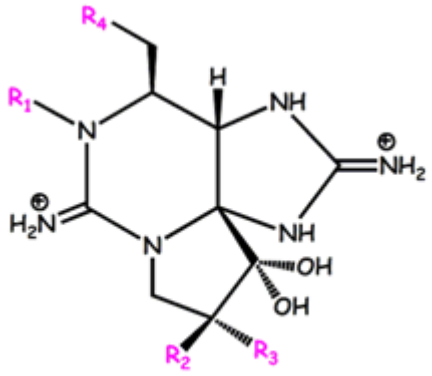
Figure 2. Map showing the production areas on the north and west coasts of Iceland in 2009.

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

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

Figure 5. Comparison of Alexandrium Cell Counts in the water (cells/L) and total sample toxicity of the harvested mussels (µg STX diHCl eq./kg) returned by both LC-FLD and MBA in Eyjafjordur and Breidafjordur.

Figure 6. Percentage PST profiles determined by LC-FLD (in terms of µmol/kg) from mussel samples collected from Eyjafjordur and Breidafjordur.



Carbamate toxins

R_4 = -OOC-NH_2

N-Sulfocarbamoyl toxins

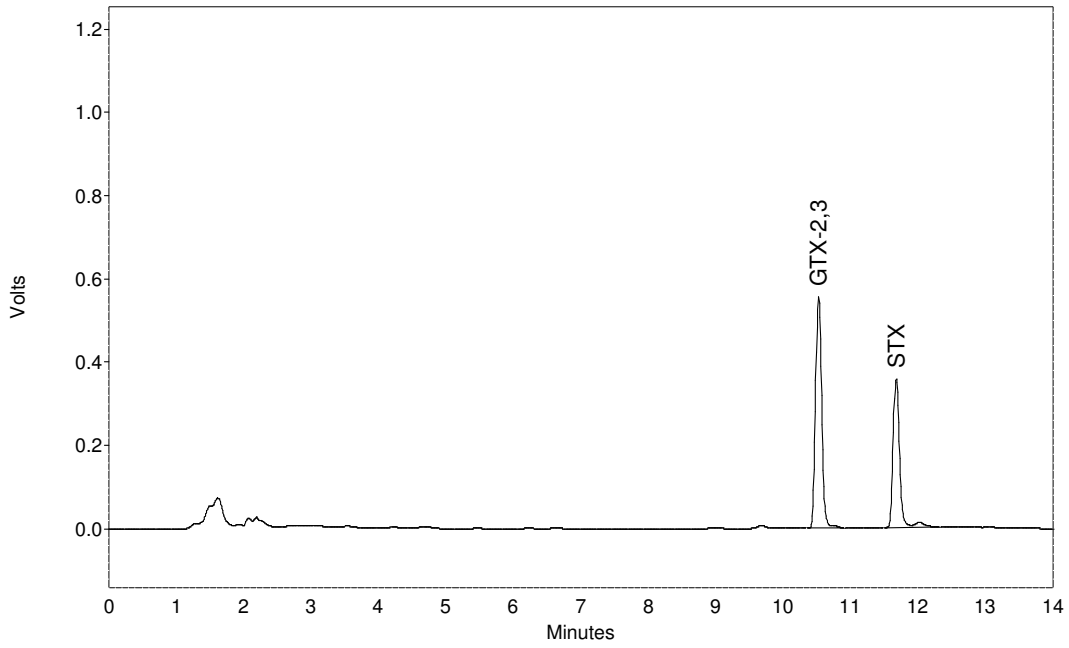
R_4 = -OOC-NH-SO_3^-

Decarbamoyl toxins

R_4 = -OH

Figure 1

Figure 2



564
565 **Figure 3**
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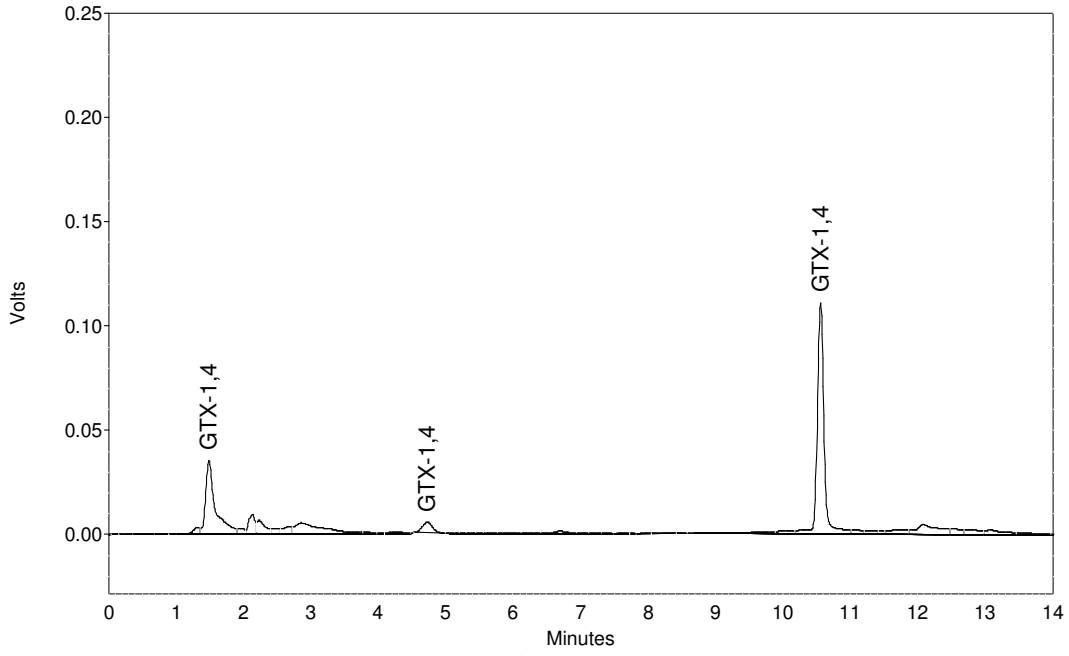

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571 **Figure 4**
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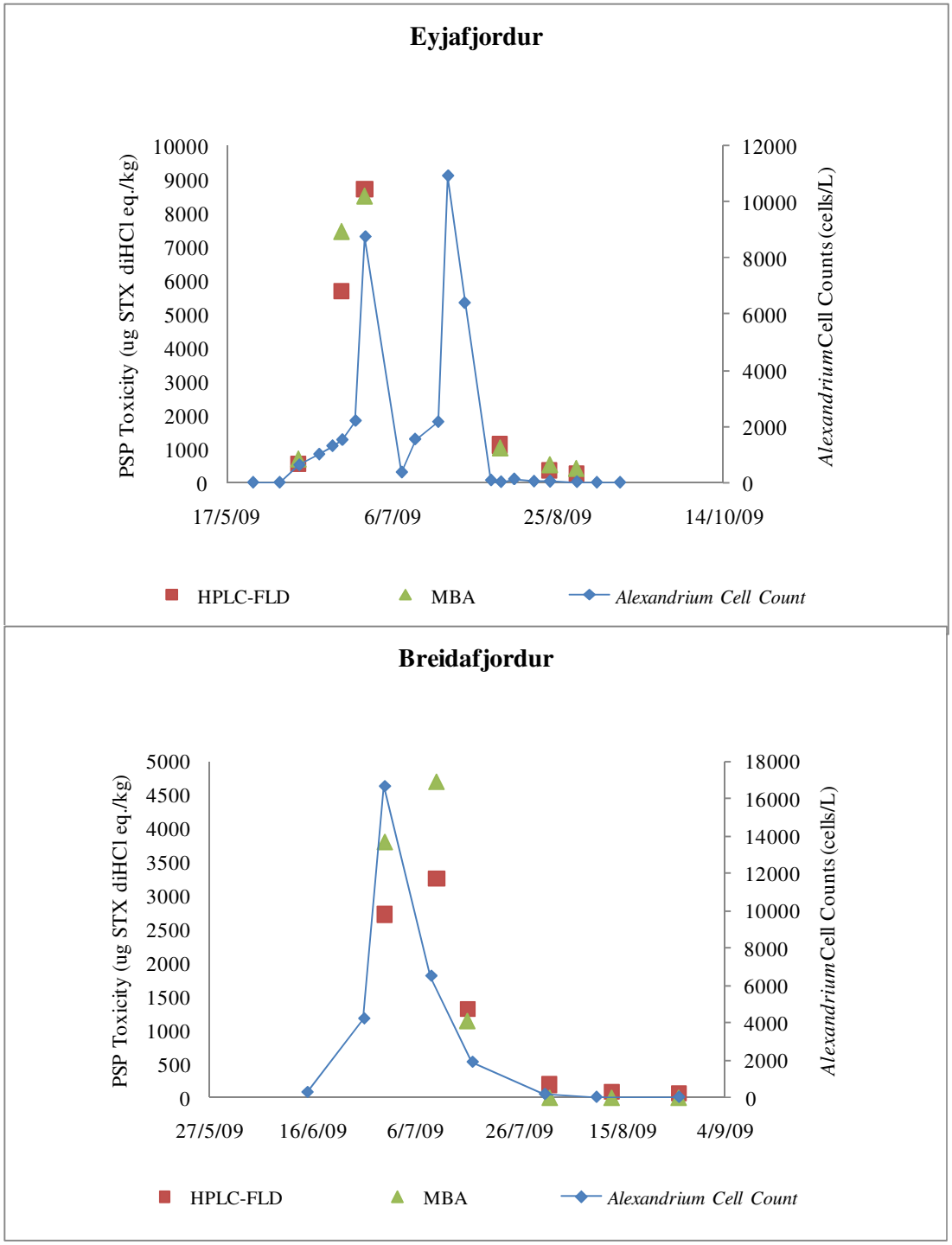
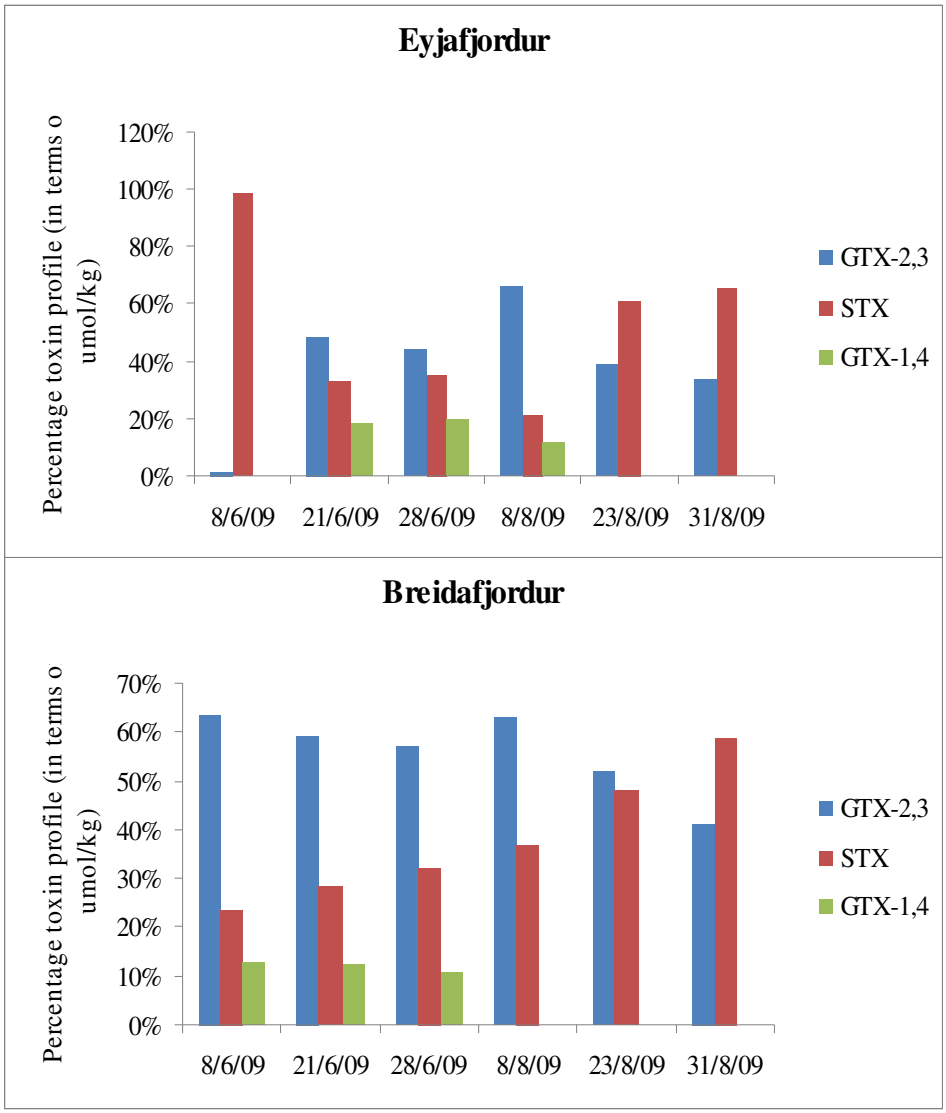

Eyjafjordur
PSP Toxicity (ug STX diHCl eq./kg)
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
12000
10000
8000
6000
4000
2000
0
Alexandrium Cell Counts (cells/L)
17/5/09
6/7/09
25/8/09
14/10/09
HPLC-FLD
MBA
Alexandrium Cell Count
Breidafjordur
PSP Toxicity (ug STX diHCl eq./kg)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
Alexandrium Cell Counts (cells/L)
27/5/09
16/6/09
6/7/09
26/7/09
15/8/09
4/9/09
HPLC-FLD
MBA
Alexandrium Cell Count

Figure 5



585
586 **Figure 6**
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List of Tables

Table 1

Sample	Sampling Date	Cell Counts (Alex spp.) cells/L	
		Eyjafjordur	Breidafjordur (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	-

(-) no samples taken

Table 2

Sample	Sampling Date	Concentration (µmol/kg)			Total Toxicity µgSTXdiHCl-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

n.d. Toxin not detected
Gonyautoxin (GTX), saxitoxin (STX)
LOQ for MBA 280 µgSTXdiHCl-eq./kg

705 **Table 3**

Sample	Sampling Date	Concentration (µmol/kg)			Total Toxicity µgSTXdiHCl-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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