



## First detection of paralytic shellfish poisoning (PSP) toxins in Icelandic mussels (*Mytilus edulis*): Links to causative phytoplankton species

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### ARTICLE INFO

#### Article history:

Received 2 August 2012

Received in revised form

25 September 2012

Accepted 2 October 2012

#### Keywords:

Paralytic shellfish poisoning (PSP)

*Alexandrium*

Iceland

Liquid chromatography (LC)

Mouse bioassay (MBA)

### 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 *Alexandrium tamarense*, with smaller numbers of *Alexandrium 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.

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### 1. Introduction

Paralytic shellfish poisoning (PSP) is caused by a group of 58 closely related compounds (Wiese, D'Agostino, Mihali, Moffitt, & Neilan, 2010) based on a tetrahydropurine skeleton (Fig. 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 (Dell'Aversano, Eaglesham, & Quilliam, 2004; Onodera, Satake, Oshima, Yasumoto, & Carmichael, 1997). 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 (Garcia, del Carmen Bravo, Lagos, & Lagos, 2004; Gessner et al., 1997; IPCS, 1984; Llewellyn et al., 2002; Shumway, 1990). 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, p. 5).

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

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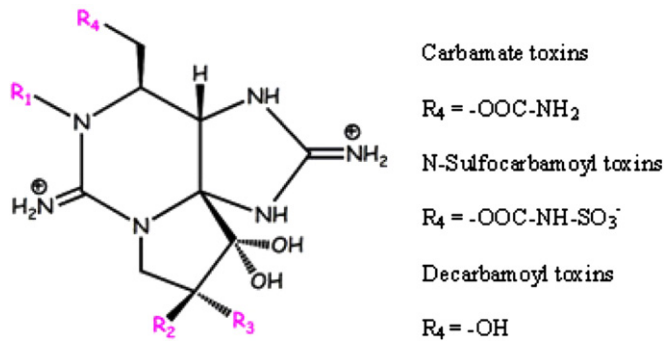


Fig. 1. Structure of the PSP toxins.

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  $\mu\text{g}$  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, Eydal, Gunnarsson, Gudmundsson, & Valsdóttir, 2010, p. 6). 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, p. 6).

In this report we present data from the analysis of whole flesh mussel (*M. edulis*) samples collected from two of these fjords located on the west and north coasts of Iceland during a bloom of *Alexandrium* spp. in 2009. Samples were analysed for PSP toxicity by MBA with additional confirmatory analysis carried out by LC-FLD to determine toxin profiles and total saxitoxin equivalents. The contamination of blue mussels with PSTs in Iceland in 2009 represents a new and unique geographical location for the occurrence of these toxins, and one which may potentially result in

a serious impact upon the livelihood of Icelandic shellfish producers and exporters.

## 2. Methods and materials

### 2.1. Chemicals

All chemicals and solvents used were of analytical or HPLC grade. The water was supplied from a reverse osmosis system (Barnstead Int., Dubuque, IA, USA). Acetic acid, hydrochloric acid, ammonium formate, ammonium acetate, sodium chloride, sodium hydroxide, hydrogen peroxide, disodium hydrogen phosphate and periodic acid were purchased from Sigma–Aldrich (Steinheim, Germany). Acetonitrile was purchased from Labscan (Stillorgan, Ireland). Certified reference toxins: gonyautoxin 1 and 4 (GTX-1,4), neosaxitoxin (NEO), decarbamoylsaxitoxin (dcSTX), gonyautoxin 2 and 3 (GTX-2,3), gonyautoxin 5 (GTX-5), N-sulfocarbamoyl-gonyautoxin 2 and 3 (C-1,2), decarbamoylneosaxitoxin (dcNEO), decarbamoyl-gonyautoxin 2 and 3 (dcGTX-2,3) and saxitoxin (STX) were obtained from the Institute of Marine Biosciences, National Research Council Canada (IMB, NRCC, Halifax, Nova Scotia, Canada). The certified reference materials (CRMs) were first diluted in water (adjusted to  $\text{pH } 4 \pm 0.1$  with 0.1 M acetic acid) to prepare primary stock solutions. Further dilutions were performed in 0.1 mM 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 (Fig. 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  $\mu\text{m}$  mesh. The net was hauled from a depth of 5 m 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 h according to the Utermöhl method (Hasle, 1978) and examined in an inverted microscope where toxic species were identified and counted (Gudfinnsson et al., 2010, p. 6).

#### 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  $< -20^\circ\text{C}$  until frozen samples were dispatched in one batch to the Marine Institute Ireland on ice.

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

#### 2.2.3. MBA analysis

The MBA analysis involved acidic aqueous extraction in 0.1 M HCl. Aliquots (1 ml) were injected intraperitoneally into male albino CD1 strain mice in triplicate and toxicity ( $\mu\text{gSTXdiHCl-eq/kg}$ ) was calculated from median death times using Sommer's tables.



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

The method was standardised using a certified reference standard of STX obtained from the Institute of Marine Biosciences, National Research Council Canada (IMB, NRCC, Halifax, Nova Scotia, Canada). The MBA procedure was carried out following AOAC official method 959.08 (Anon, 2005a).

#### 2.2.4. Solid phase extraction (SPE)

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

#### 2.2.5. LC–FLD analysis

The extraction, oxidation and analysis steps were carried out closely following the official method AOAC 2005.06. A Shimadzu (Kyoto, Japan) HPLC system with a 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 × 4.6 mm, 5 μm) 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. Breidafjörður

Between the years 2005–2007, in Breidafjörður (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, p. 6) 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 Breidafjörður (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 16,000 cells/L (Table 1).

##### 3.1.2. Eyjafjörður

At Hrisey Island in Eyjafjörður, *Alexandrium* spp. have been observed each year from 2005 to 2008 with cell densities >6000 cells/L found in 2005 (data not shown). In 2009 *Alexandrium* spp. peaked twice, firstly at over 8000 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 *Alexandrium*

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

Sample	Sampling date	Cell counts ( <i>Alexandrium</i> 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	–	16,680
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	10,920	–
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.

*tamarensis* with small numbers of *Alexandrium 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  $\mu\text{gSTXdiHCl-eq/kg}$ .

#### 3.2.2. Eyjafjordur

The MBA and LC–FLD toxicity data generated from the analysis of the Eyjafjordur mussel samples collected during 2009 is

**Table 2**  
MBA and LC–FLD data of mussel (*M. edulis*) samples harvested from Breidafjordur, Iceland.

Sample	Sampling date	Concentration ( $\mu\text{mol/kg}$ )			Total toxicity $\mu\text{gSTXdiHCl-eq./kg}$	
		GTX-2,3	STX	GTX-1,4	LC-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  $\mu\text{gSTXdiHCl-eq./kg}$ .

**Table 3**  
MBA and LC–FLD data of mussel (*M. edulis*) samples harvested from Eyjafjordur, Iceland.

Sample	Sampling date	Concentration ( $\mu\text{mol/kg}$ )			Total toxicity $\mu\text{gSTXdiHCl-eq./kg}$	
		GTX-2,3	STX	GTX-1,4	LC-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

n.d. Toxin not detected.

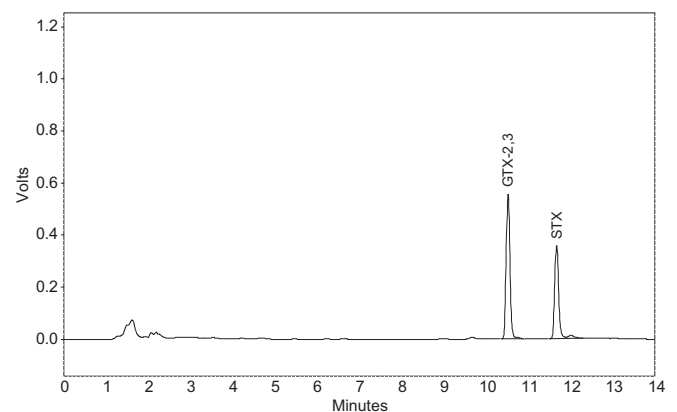
Gonyautoxin (GTX), saxitoxin (STX).

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

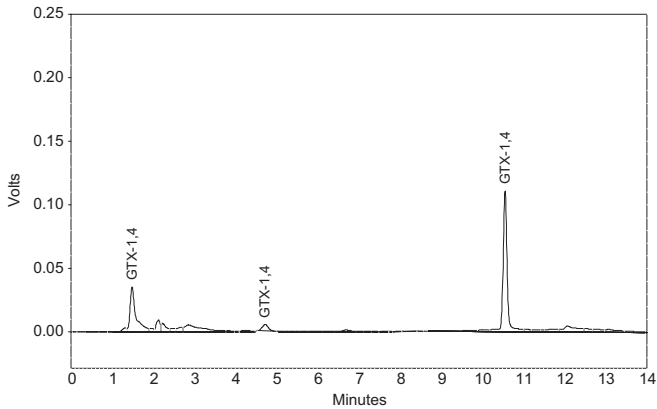
Chromatograms taken after peroxide and periodate oxidation of a sample from Breidafjordur are presented in Figs. 3 and 4. Results from both fjords showed the absence of any other toxin oxidation product peaks which may relate to other PSP toxins or metabolic products. Analysis of unoxidised extracts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

## 4. Discussion

Conditions within both fjords during the sampling periods were favourable for phytoplankton growth as confirmed through the data presented in Table 1, where cell counts of *Alexandrium* spp. reached record levels in both Eyjafjordur and 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, p. 6). 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

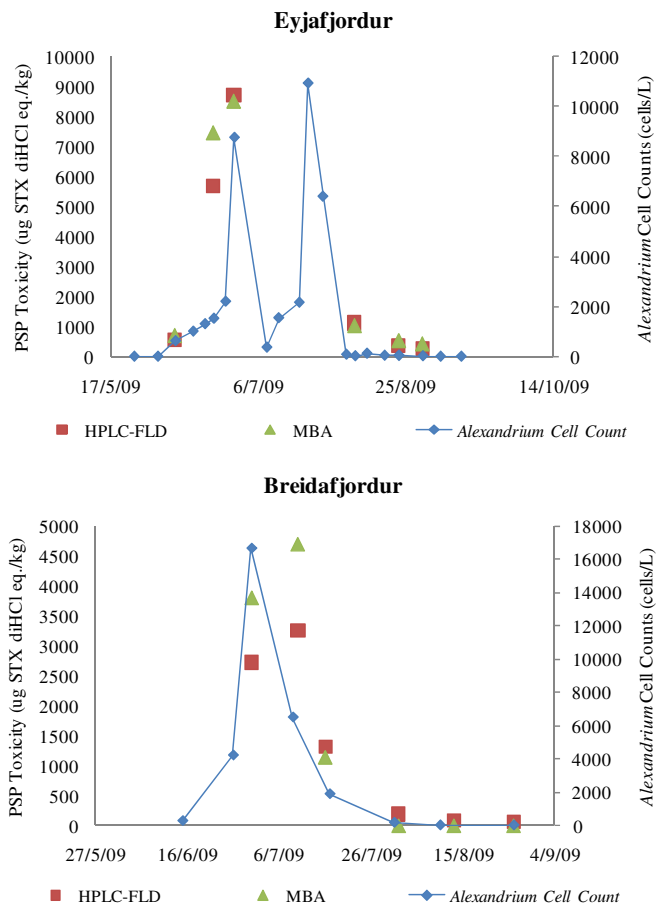


**Fig. 3.** LC–FLD separation of PSP toxins present in an Icelandic sample from Breidafjordur in 2009 after SPE-C18 cleanup and peroxide oxidation.



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

cyclic variations such as oscillations to the North Atlantic subpolar gyre (Hátún, Payne, & Jacobsen, 2009; Hátún, Sando, Drange, Hansen, & Valdimarsson, 2005). 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, p. 6).



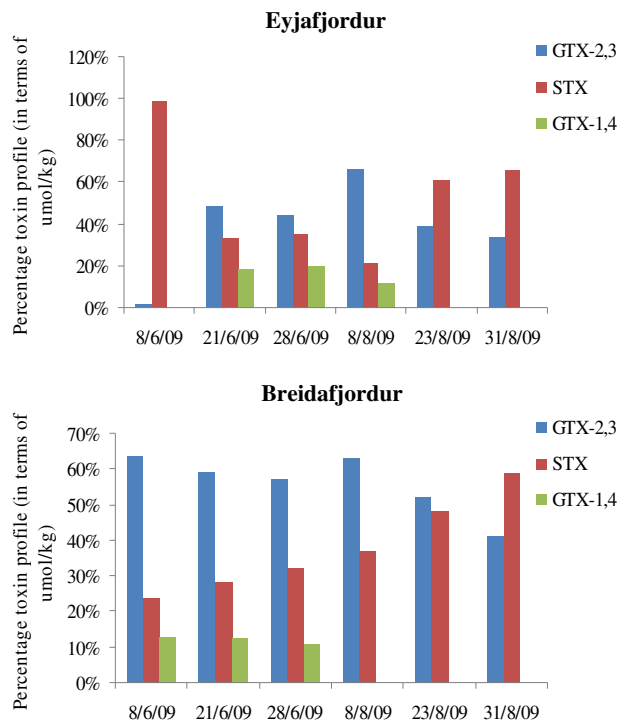
**Fig. 5.** Comparison of Alexandrium cell counts in the water (cells/L) and total sample toxicity of the harvested mussels ( $\mu\text{gSTXdiHCl eq./kg}$ ) returned by both LC-FLD and MBA in Eyjafjörður and Breidafjörður.

A comparison of the results obtained from both the algal cell counts and the toxicity tests are illustrated in Fig. 5. A clear correlation is evident between the high cell counts recorded and flesh samples containing higher concentrations of PSTs. Notably, the data from Breidafjörður 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 Eyjafjörður 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 2005.06) and are therefore presented as a combined sum using the higher toxicity factor of the two co-eluted epimers to calculate total toxicity. Through analysis using this method the toxin profile was determined and found to be similar in both fjords with samples predominated by the carbamate toxins GTX-2,3. STX was the next most abundant toxin present with GTX-1,4 observed in half the samples analysed (Fig. 6).

The toxin profiles determined in these samples are similar to those found in other areas where *Alexandrium* spp. predominates such as the UK (Turrel, Lacaze, & Stobo, 2007) where the toxins GTX-2,3 and STX predominate with lower levels of GTX-1,4, NEO and GTX-5 also being found, or in Ireland where GTX-2,3 has been found to predominate with lower relative concentrations of STX and GTX-1,4 being determined (Furey, James, & Sherlock, 1998; Marine Institute Ireland internal data). Interestingly, there is no



**Fig. 6.** Percentage PST profiles determined by LC-FLD (in terms of  $\mu\text{mol/kg}$ ) from mussel samples collected from Eyjafjörður and Breidafjörður.

indication of the presence of any of the N-sulfocarbamoyl toxins such as C-1,2, which have been found to occur in mussels containing PSP toxins in some UK waters since 2008 (Turner, personal communication) and which are associated with a number of different strains of *Alexandrium* spp. The Norwegian PSP toxin profile typically observed is slightly different to that observed in Iceland, being predominated by GTX-1,4, with both NEO and STX being found at lower relative concentrations (Sayfritz, Aasen, & Aune, 2008). The differences between profiles in the region and Iceland's are mainly the absence of the toxins NEO and C-1,2 from samples analysed.

Profiles of *A. tamarense* mainly consist of the N-sulfocarbamoyl toxins, C-1,2 and the high potency carbamate toxins GTX-1-4, NEO and STX (Ichimi, Suzuki, & Ito, 2002; Persich, Kulis, Lilly, Anderson, & Garcia, 2006). Profiles of *A. ostenfeldii* can contain the spirolides as well as the PSTs GTX-6, C-1,2 and GTX-2,3 (Ciminiello et al., 2006; Hansen, Cembella, & Moestrup, 1992). The absence of the N-sulfocarbamoyl toxins C-1,2 from mussel samples taken from both harvesting areas, if not relating to the toxin profile in the source algae, could instead be due to the metabolic conversion of these toxins in shellfish to GTX-2,3 via desulfonation and epimerization (Krock, Seguel, & Cembella, 2007).

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

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

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

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

#### 4.2. Chemical and biological method analysis

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

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

The level of observed time delay between the peaks in phytoplankton cell presence found in the water and the maximum levels of toxicity found in shellfish (Fig. 5) is also of interest. At Breidafjordur, the peak in toxicity appears approximately two weeks after the measured maximum of *Alexandrium* cells. This observation is consistent with those observed previously from water and flesh samples collected in the St. Lawrence region, Canada (Blasco, Lefebvre, Bonneau, Gelinas, & Packard, 2003) or from Busta Voe Lee North, Scotland (CEFAS Contract Report C2649) where time delays of over 7 days have been found.

## 5. Conclusions

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

## Acknowledgements

The assistance of the biotoxin team at the Irish Marine Institute in the analysis of shellfish samples is gratefully acknowledged. This work was carried out in conjunction with the Icelandic Ministry of Fisheries and Food who generously provided shellfish samples and phytoplankton data.

## References

- Anon. (2004). Commission regulation (EC) No. 853/2004 of 29th April 2004 of the European Parliament and of the Council of 29th April 2004 laying down specific hygiene rules for food of animal origin. *Official Journal of the European Union*, L226, 22–80.
- Anon. (2005a). AOAC official method 959.08. Paralytic shellfish poison. Biological method. Final action. In M. W. Truckses (Ed.), *AOAC official methods of analysis* (18th ed.). Chapter 49: Natural toxins, (pp. 79–80) Gaithersburg, MD, USA: AOAC International.
- Anon. (2005b). *AOAC official method 2005.06 quantitative determination of paralytic shellfish poisoning toxins in shellfish using pre-chromatographic oxidation and liquid chromatography with fluorescence detection* (18th ed.). Gaithersburg, MD, USA: AOAC International.

- Anon. (2006). Commission regulation (EC) No 1664/2006 of 6th Nov. 2006 amending regulation (EC) No 2074/2005 as regards implementing measures for certain products of animal origin intended for human consumption and repealing certain implementing measures. *Official Journal of the European Union*, L320, 13–45.
- Blasco, D., Levasseur, M., Bonneau, E., Gelinas, R., & Packard, T. T. (2003). Patterns of paralytic shellfish toxicity in the St. Lawrence region in relationship with the abundance and distribution of *Alexandrium tamarense*. *Scientia Marina*, 67(3), 261–278.
- Catterall, W. A., Cestè le, S., Yarov-Yarovoy, V., Yu, F. H., Konoki, K., & Scheuer, T. (2007). Voltage-gated ion channels and gating modifier toxins. *Toxicon*, 49, 124–141.
- CEFAS Contract Report – C2649. Biotxin monitoring report for Scotland. Monitoring for paralytic shellfish poisoning toxins and lipophilic toxins – 1st April 2006 to 31st March 2007 final report on behalf of the food standards agency Scotland contract reference: PAU 179 – S02007/PSP & DSP.
- Ciminiello, P., Dell'Aversano, C., Fattorusso, E., Magno, S., Tartaglione, L., Cangini, M., et al. (2006). Toxin profile of *Alexandrium ostenfeldii* (Dinophyceae) from the Northern Adriatic Sea revealed by liquid chromatography-mass spectrometry. *Toxicon*, 47, 597–604.
- Dell'Aversano, C., Eaglesham, G. K., & Quilliam, M. A. (2004). Analysis of cyanobacterial toxins by hydrophilic interaction liquid chromatography – mass spectrometry. *Journal of Chromatography A*, 1028(1), 155–164.
- FAO/IOC/WHO (Food and Agriculture Organization of the United Nations/Intergovernmental Oceanographic Commission of UNESCO/World Health Organization). (2004). *Background document of the Joint FAO/IOC/WHO ad hoc expert consultation on biotoxins in Bivalve Molluscs*. Oslo, Norway, September 26–30, 2004.
- Fast, M. D., Cembella, A. D., & Ross, N. W. (2006). In vitro transformation of paralytic shellfish toxins in the clams *Mya arenaria* and *Protothaca staminea*. *Harmful Algae*, 5, 79–90.
- Furey, A., James, K. J., & Sherlock, I. R. (1998). First report of paralytic shellfish poisoning toxins in the republic of Ireland. In B. Reguera, J. Blanco, M. L. Fernandez, & T. Wyatt (Eds.), *Harmful algae* (pp. 70–71). Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO.
- García, C., del Carmen Bravo, M., Lagos, M., & Lagos, N. (2004). Paralytic shellfish poisoning: post-mortem analysis of tissue and body fluid samples from human victims in the Patagonia fjords. *Toxicon*, 43(2), 149–158.
- Gessner, B. D., Bell, P., Doucette, G. J., Moczydlowski, E., Poli, M. A., Van Dolah, F., et al. (1997). Hypertension and identification of toxin in human urine and serum following a cluster of mussel-associated paralytic shellfish poisoning outbreaks. *Toxicon*, 35(5), 711–722.
- Gudfinnsson, H. G., Eydal, A., Gunnarsson, K., Gudmundsson, K., & Valsdóttir, K. (2010). *Monitoring of toxic phytoplankton in three Icelandic fjords*. ICES theme session N, ICES CM 2010/N:12.
- Hansen, P. J., Cembella, A. D., & Moestrup, Ø. (1992). The marine dinoflagellate *Alexandrium ostenfeldii*: paralytic shellfish toxin concentration, composition and toxicity to a tintinnid ciliate. *Journal of Phycology*, 28, 597–603.
- Hasle, G. R. (1978). Using the inverted microscope. In A. Sourina (Ed.), *Phytoplankton manual* (pp. 191–196). Paris: UNESCO.
- Hátún, H., Payne, M. R., & Jacobsen, J. A. (2009). The North Atlantic subpolar gyre regulates the spawning distribution of blue whiting (*Micromesistius poutassou*). *Canadian Journal of Fisheries and Aquatic Sciences*, 66, 759–770.
- Hátún, H., Sando, A. B., Drange, H., Hansen, B., & Valdimarsson, H. (2005). Influence of the Atlantic subpolar gyre on the thermohaline circulation. *Science*, 309, 1841–1844. <http://dx.doi.org/10.1126/science.1114777>, Washington, D.C. 16166513
- Iceland seafood market report, June 2010. Islandsbanki, Reykjavik, Iceland. <http://www.islandsbanki.is/servlet/file/store156/item64129/version2/Seafood%20report%202010%2005%20vef.pdf>. Accessed 21.01.11.
- Icelandic Fisheries. Information centre of the Icelandic Ministry of Fisheries and Agriculture. <http://www.fisheries.is/aquaculture/species/blue-mussel/>. Accessed 21.01.11.
- Ichimi, K., Suzuki, T., & Ito, A. (2002). Variety of PSP toxin profiles in various culture strains of *Alexandrium tamarense* and change of toxin profile in natural *A. tamarense* population. *Journal of Experimental Marine Biology and Ecology*, 273, 51–60.
- IPCS (International Programme on Chemical Safety). (1984). Aquatic (marine and freshwater) biotoxins. In *Environmental health criteria*, Vol. 37. World Health Organization, ISBN 92 4 154097 4.
- Krock, B., Seguel, C. G., & Cembella, A. D. (2007). Toxin profile of *Alexandrium catenella* from the Chilean coast as determined by liquid chromatography with fluorescence detection and liquid chromatography coupled with tandem mass spectrometry. *Harmful Algae*, 6, 734–744.
- Llewellyn, L., Dodd, M. J., Robertson, A., Ericson, G., deKoning, C., & Negri, A. P. (2002). Post-mortem analysis of samples from a human victim of a fatal poisoning caused by the xanthid crab, *Zosimus aeneus*. *Toxicon*, 40, 1463–1469.
- Onodera, H., Satake, M., Oshima, Y., Yasumoto, T., & Carmichael, W. W. (1997). New saxitoxin analogues from the freshwater filamentous cyanobacterium *Lyngbya wollei*. *Journal of Natural Toxins*, 5, 146–151.
- Persich, G. R., Kulis, D. M., Lilly, E. L., Anderson, D. M., & Garcia, V. M. T. (2006). Probable origin and toxin profile of *Alexandrium tamarense* (Lebour) Balech from southern Brazil. *Harmful Algae*, 5, 36–44.
- Quilliam, M. A. (April 2007). *Supplemental information for PSP toxin CRMs: Structures, molecular weights, concentrations and toxicities*. CRMP technical report CRM-PSP-20070411. Halifax: National Research Council Canada.
- Sayfritz, S. J., Aasen, J. A. B., & Aune, T. (2008). Determination of paralytic shellfish poisoning toxins in Norwegian shellfish by liquid chromatography with fluorescence and tandem mass spectrometry detection. *Toxicon*, 52, 330–340.
- Scientific Opinion of the Panel on contaminants in the food chain on a request from the European Commission on Marine Biotoxins in Shellfish – Saxitoxin Group. (2009). *The EFSA Journal*, 1019, 1–76.
- Shumway, S. E. (1990). A review of the effects of algal blooms on shellfish and aquaculture. *Journal of the World Aquaculture Society*, 21, 65–104.
- Turner, A. D., Norton, D. M., Hatfield, R. G., Morris, S., Reese, A. R., Algoet, M., et al. (2009). Refinement and extension of AOAC method 2005.06 to include additional toxins in mussels: single-laboratory validation. *Journal of AOAC International*, 92(1), 190–207.
- Turrel, E. A., Lacaze, J. P., & Stobo, L. (2007). Determination of paralytic shellfish poisoning (PSP) toxins in UK shellfish. *Harmful Algae*, 6, 438–448.
- Wiese, M., D'Agostino, P. M., Mihali, T. K., Moffitt, M. C., & Neilan, B. A. (2010). Neurotoxic alkaloids: saxitoxin and its analogues. *Marine Drugs*, 8, 2185–2211.