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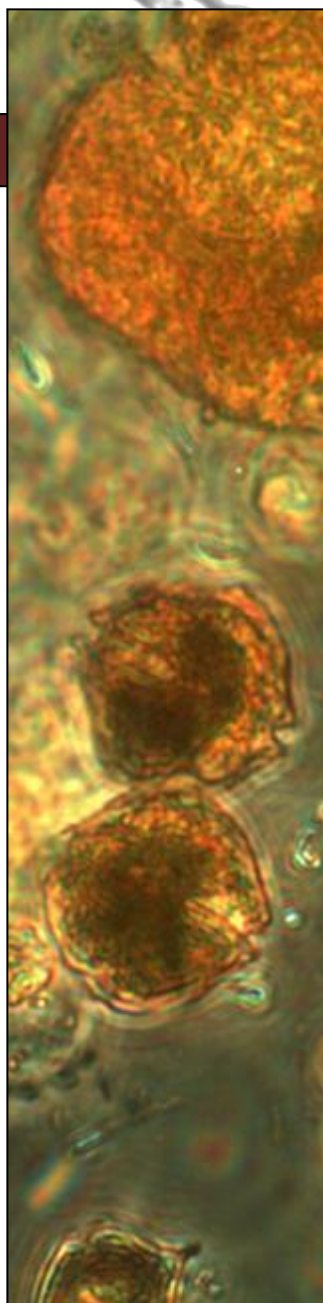
Development of a monitoring programme for the occurrence of phytoplankton derived toxins in blue mussels (*Mytilus edulis*) in northern Icelandic coastal waters

- Report for VSS 2011-2012



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Report Summary

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Title	*Development of a monitoring programme for the occurrence of phytoplankton derived toxins in blue mussels (Mytilus edulis) in northern Icelandic coastal waters*	
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Summary: *Alexandrium* spp., *Dinophysis* spp. and *Pseudo-nitzschia* spp. are known to produce different classes of toxins, which are bio-accumulated in filter feeders such as the blue mussel *Mytilus edulis* during ingestion. If theses mussels are consumed by humans, these toxins can cause different illnesses and in some cases lead to death. Due their mode of action these toxins are classified as amnesic, paralytic and diarrheic shellfish toxin groups - short ASP, DSP and PSP. In order to gain basic data of the appearance of such toxin producing taxa in northern Icelandic coastal waters, two different stations in this region (Miðfjörður and Skagaströnd) were chosen and monitored during spring, summer and autumn in 2011 and 2012. In particular, abiotic parameters such as temperatures and salinities, taxa abundances as well as the presence of ASP, DSP and PSP toxins in mussel flesh samples were continuously recorded during this time. Additionally in 2011 macronutrients as well as the presence of ASP, DSP and PSP toxins were analysed in the water column in 2011, using so-called solid-phase adsorption toxin tracking (SPATT) bags. Beside the use of different commercial biotoxin-tests (Jellett® test, the Biosense® Laboratories ASP test, the DSP OkaTest® ZEU-IMMUNOTEC and the Ridascreen® Fast PSP test) for screening purposes, samples were also analysed by liquid chromatography to gain information about the toxin profiles present in the samples. In preparation for the qPCR method development, first isolation attempts of Icelandic *Alexandrium tamarense* strains as well as the cultivation of Scottish PSP toxin and non-toxin producing strains of this species complex were done. Regarding the results of the cell counts in relation to the accumulated toxins, a clear trend to the predictably of the toxin occurrences after a bloom event of the potentially toxin producing taxa was observed in 90% of the cases. Moreover, the results of the toxin tests gave evidence of the presence of ASP, DSP and PSP toxins in the water column (2011) as well as in mussel flesh samples (2011/2012), showing for ASP and DSP overall a good accordance between the ELISA/enzyme assays and the LC analysis. Although a high accordance between the ASP and DSP toxin tests and LC measurements was calculated by regression analysis, it has to be emphasised that all tests and analysis in this study were only conducted once without repetition. The causative factor for the occurrence of toxins and potential toxin producing taxa was not identified. Thus, further detailed investigations are needed to relate environmental data, species identification and toxin profiles to an overall picture of the environment. Selected data will be published in a scientific peer reviewed journal.

Keywords: *Blue Mussel, potentially toxin producing phytoplankton taxa, marine biotoxin analyses, monitoring*

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1. Introduction

1.1 Marine Phytoplankton Blooms and their Harmful Potential for Humans

Algal blooms, or higher than normal concentrations of phytoplankton (greek: φυτόν (*phyton*), meaning "plant", and πλαγκτός (*planktos*), meaning "wanderer" or "drifter"), occur when environmental conditions allow phytoplankton growth to exceed losses due to mortality, sinking, grazing, etc.; or when conditions result in the physical concentration of phytoplankton cells. Furthermore, harmful algal blooms (HABs) are phytoplankton blooms that are in some way deleterious to humans or the environment. Algal species may be considered “harmful” for many different reasons, and the harmful effects are not necessarily dependent on high biomass or cellular abundances (Smayda 1997). It is generally perceived that there have been more coastal harmful algal blooms, often of greater geographic extent and/or longer duration, with more toxic species observed, more fisheries affected, and higher associated costs from HABs in the past decade than in previous decades worldwide (Anderson 1989, Smayda 1990, Hallegraeff 1993, Anderson et al. 2002, Glibert et al. 2005).

1.1.1 Ecology of Harmful Phytoplankton Blooms (HABs)

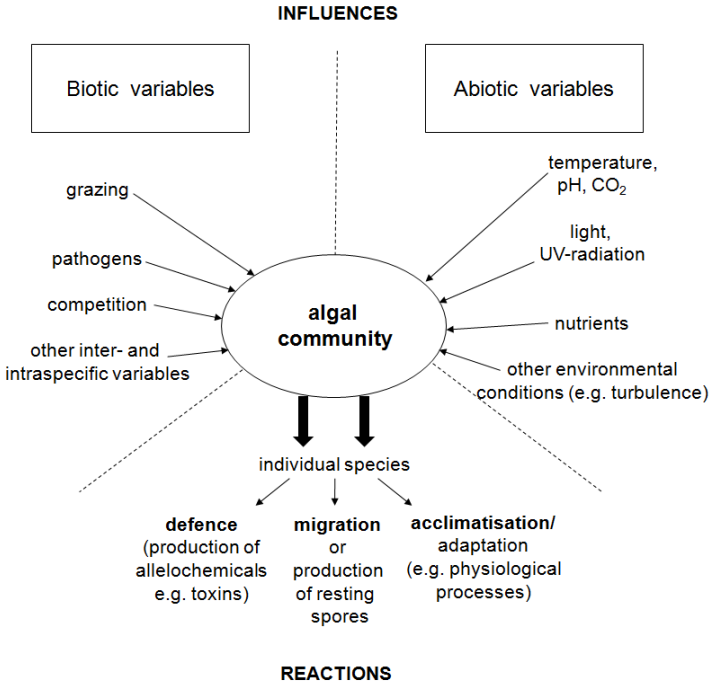


Figure 1: One dimensional schematic diagram of the potential abiotic and biotic influences on algal species.

In most cases the cause effects for the occurrence of HABs are not clear (Davidson et al. 2012, Gowen et al. 2012). More than one cause is frequently involved in this phenomenon, and the causes for global expansion of a given species can differ among species. While strong relationships have been shown for many years in freshwater ecosystems between phosphorus loadings and harmful

cyanobacteria blooms (Schindler 1977, Burkholder 2002) and, although, there are numerous examples worldwide of increases in HABs which seemed to be linked to increase nutrient loading (Smayda 1990, 1997, Anderson et al. 2002, Glibert et al. 2005 a, b, Glibert & Burkholder 2006), no overall consensus regarding the role of anthropogenic nutrients in stimulating the occurrence of HABs was found (Gowen et al. 2012).

Specific species or species groups have numerous physiological acclimatisation/adaptations that permit them to cope to abiotic environmental factors differentially. For example, temperature plays a crucial role in the bloom dynamics of the cyst-forming dinoflagellates such as *Alexandrium tamarense* (Lebour) Balech as well as raphidophytes and many cyanobacteria, which have well-defined seasonal temperature windows. Another major factor governing the growth of different species of phytoplankton is the availability of mineral and/or organic nutrients. It is widely accepted that in coastal waters, it is the availability of dissolved inorganic N as ammonium (NH^+_4), nitrate (NO^-_3) and nitrite (NO^-_2) that is most likely to constrain (limit) phytoplankton growth (Ryther & Dunstan 1971, Howarth & Marino 2006). Especially, distinctions in species groups display preferences for specific nutrient regimes, including nutrient ratio or form (Smayda 1990, 1997, Anderson et al. 2002, Smayda & Reynolds 2001, 2003, Glibert & Burkholder, 2006). For instance, most HAB species (e.g. dinoflagellates) have been demonstrated to have either some capability of mixotrophy/organic nutrient uptake or a requirement for micronutrients (Graneli & Turner 2006). Also correlations of the domoic acid (DA) producing diatom *Pseudo-nitzschia* Paragallo and silicic (Si), which is the main part of their cell walls in form of hydrated silicon dioxide, were found. With increased nutrient inputs from runoff come reduced Si:N and Si:P ratios (reviewed in Anderson et al. 2002), a factor shown to contribute to and/or be associated with increases of *Pseudo-nitzschia* abundance and DA production (Pan et al. 1996, Fehling et al. 2004, Parsons & Dortch 2002, Anderson et al. 2006). Whereas most diatoms only thrive under Si-replete conditions, some *Pseudo-nitzschia* species appear to be favoured by Si-limitation and certainly tend towards toxicity under extreme Si-limitation (Pan et al. 1996, 1998, Bates et al. 1998). In conclusion, physical (e.g. rising temperatures, acidification, turbidity), biological (e.g. competition, grazing, pathogens), and other factors (e.g. allelopathy) may modulate harmful algal species' responses to nutrient loadings (e.g. Vadstein et al. 2004, Gobler et al. 2002, Sellner et al. 2003, Glibert et al. 2005a, Fig. 1).

However, once cells of these species enter the water column, other factors such as nutrients, turbulence, and grazing determine the outcome of competition (Hallegraeff 2010). In addition, climate change, overfishing and increased aquaculture alter food webs which may, in turn, alter the community of grazers that feed on HABs (Heisler et al. 2008). Finally, for large-biomass HABs, the hypothesis that nutrient enrichment can cause HABs is supported in some water bodies but not in others. The global evidence that enrichment brings about an increase in low-biomass HABs of toxin-producing species is more equivocal it is concluded that evidence of a link in one coastal region should not be taken as evidence of a general linkage in other coastal regions (Gowen et al. 2012).

1.1.2 Impacts of HABs on Human Health and Economy

Marine biotoxins detected worldwide, but particularly in European waters, were originally classified based on their acute symptomatic effect in humans following intoxification. The three main groups monitored in the European Union (EU) are:

- Amnesic Shellfish Poisoning (ASP);

- Paralytic Shellfish Poisoning (PSP) toxins; and,
- Diarrheic Shellfish Poisoning (DSP) toxins.

PSP toxins were detected in blue mussels (*Mytilus edulis* Linnaeus) from two harvesting areas, Eyjafjordur on the north coast and Breidafjordur on the west coast of Iceland in 2009 (Burrell et al. 2013). Dinoflagellates of the genus *Alexandrium* Halim are the most numerous and widespread saxitoxin producers and are responsible for PSP blooms in subarctic, temperate, and tropical locations. Dinoflagellates belonging to the genus *Dinophysis* Ehrenberg have been associated with the occurrence of DSP toxins in shellfish. ASP is caused by consumption of the marine biotoxin called domoic acid. This toxin is produced naturally by marine diatoms belonging to the genus *Pseudo-nitzschia* Paragallo. Generally, when accumulated in high concentrations by shellfish during filter feeding, all of these toxins can then be passed on to humans via consumption of the contaminated shellfish (reviewed by Bates and Trainer 2006, Bejarano et al. 2008, Trainer et al. 2008, Lefebvre & Robertson 2010, Bargu et al. 2011, Bargu et al. 2012, Lelong et al. 2012, Trainer et al. 2012).

The Icelandic blue mussel industry is acutely aware of the potential damage that products contaminated by marine biotoxins pose to its markets and to the safety of consumers. 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 (Burell et al. 2013). 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 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, 94 tonnes in 2011 from approximately 6 different harvesting locations (Burell et al. 2013) and 130.5 tonnes in 2012 (A. Árnadóttir, Union of Icelandic mussel farmers, pers. communication).

The monitoring of marine toxins is vital to the aquaculture industry, as these toxins may cause substantial ecological damage and economic losses through frequent or prolonged contamination and closure of harvesting sites (Hoagland & Scatasta 2006).

1.2 Relevant Toxin Producing Taxa in Icelandic Coastal Waters

1.2.1 *Pseudo-nitzschia* Paragallo

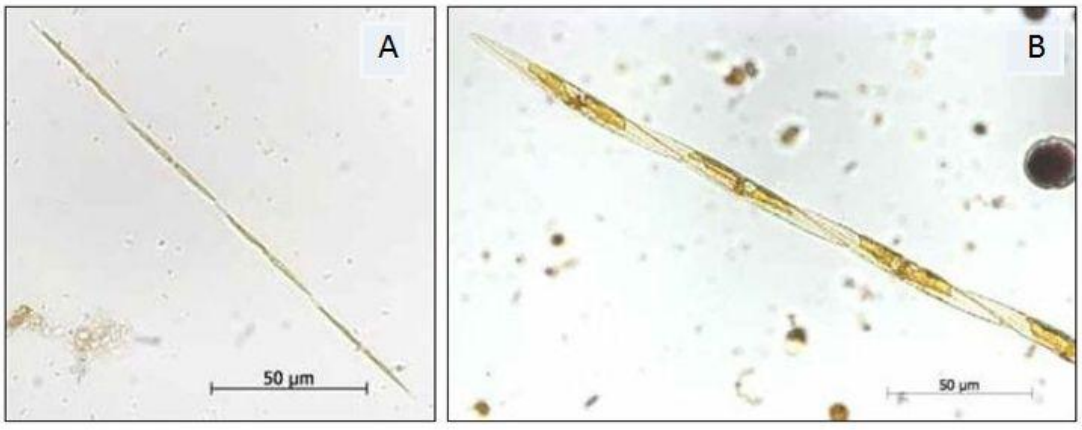


Figure 2: Diatoms of the genus *Pseudo-nitzschia* in girdle view showing overlapping cells. A) belongs to the *Pseudo-nitzschia delicatissima* type category and B) to the *Pseudo-nitzschia seriata* type (Swan & Davidson 2011).

Phytoplankton blooms consisting of toxic species of the diatom genus *Pseudo-nitzschia* are a common occurrence along the coastal areas of Iceland. Twelve species of *Pseudo-nitzschia* are now confirmed to produce DA (Trainer et al. 2010, Lundholm 2011, Trainer et al. 2012): *P. australis* Frenguelli; *P. calliantha* Lundholm, Moestrup et Hasle; or *P. cuspidata* (Hasle) Hasle emend. Lundholm, Moestrup et Hasle; *P. delicatissima* (P. T. Cleve) Heiden; *P. fraudulenta* (P. T. Cleve) Hasle; *P. galaxiae* Lundholm & Moestrup, 2002, *P. multiseries* (Hasle) Hasle; *P. multistriata* (Takano) Takano; either *P. pseudodelicatissima* (Hasle) Hasle emend. Lundholm, Hasle et Moestrup *P. pungens* (Grunow ex Cleve) Hasle; *P. seriata* (P. T. Cleve) H. Peragallo; and *P. turgidula* (Hustedt) Hasle. However, in the case of the toxic diatom *Pseudo-nitzschia* spp., recent research has identified a role for the production and release of the neurotoxic amino acid domoic acid (DA) as a part of a high efficiency iron uptake system (Wells et al. 2005). In Icelandic marine habitats the species *P. delicatissima, P. pseudodelicatissima* and *P. seriata* were monitored by the Marine Research Institute (HAFRO) on behalf of the Icelandic Food and Veterinary Authority (MAST).

1.2.2 *Dinophysis* Ehrenberg

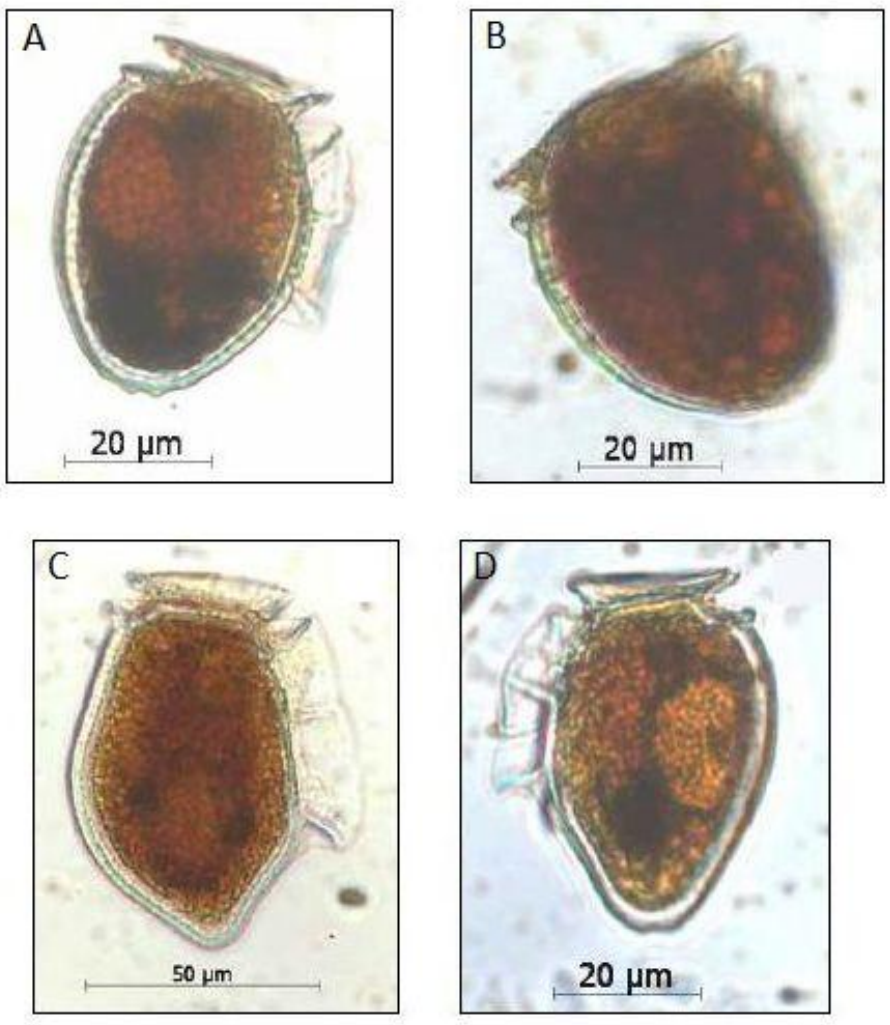


Figure 3: The four dinoflagellate species, which are monitored frequently by HAFRO in Icelandic coastal waters: A) *Dinophysis acuminata*; B) *Dinophysis rotundata*, C) *Dinophysis acuta*, and D) *Dinophysis norvegica* (Swan & Davidson 2011).

Dinoflagellates belonging to the genus *Dinophysis* have been associated with the occurrence of DSP toxins in shellfish. In Icelandic waters *D. acuminata* Claparède & Lachmann*, D. acuta* Ehrenberg*, D. norvegica* Claparède & Lachmann and *D. rotundata* Levander (= *D. arctica* Mereschkowsky) are monitored by the Marine Research Institute. Furthermore, until recently Park et al. (2008) established for the first time cultures of *Dinophysis*, some authors have performed laborious microscopic isolations of wild specimens and determined their toxin content by liquid chromatography. In several regions, different toxin profiles, as well as different cellular concentrations of toxins, have been confirmed in several *Dinophysis* species (Lee et al. 1989, Cembella, 1989, Subba Rao et al. 1993, Blanco et al. 1995, James et al. 1998, Draisci et al. 1998). Differences also have been found in algae from the same region collected in different periods of the year and in different years (Lee et al. 1989, Cembella 1989, Masselin et al. 1991, Subba Rao et al. 1993, Andersen et al. 1996, Draisci et al. 1998). These variations could explain, in part, why in different blooms of *Dinophysis* spp, toxicity concentrations found in Portuguese shellfish where different.

1.2.3 *Alexandrium* Halim

The marine dinoflagellate *Alexandrium* Halim is perhaps the most intensively studied genus of free-living planktonic dinoflagellates because of the production of potent neurotoxins associated with paralytic shellfish poisoning (PSP) (Anderson 1998, Cembella 1998, Taylor & Fukuyo 1998). The majority of toxic blooms have been caused by the morphospecies *Alexandrium catenella* (Whedon & Kofoid) Balech*, Alexandrium tamarense* (Lebour) Balech, and *Alexandrium fundyense* Balech, which together comprise the *A. tamarense* species complex. Furthermore, according to different authors, it is difficult to identify *Alexandrium* to species level in Lugol's-fixed samples, as the thecal plate structure is not clearly visible (e.g. Swan & Davidson 2011). The potentially high cellular toxicity of *A. tamarense* has led to a phytoplankton alert trigger level of the presence of a single *Alexandrium* cell in a counting chamber sample. Operationally, a density greater than 100 or 200 cells l^{-1} (equivalent to only five or ten cells in a counting chamber) might be sufficient to raise significant concerns of impending shellfish PSP positives. Knowledge of trends in the abundance of *Alexandrium* cells near shellfish farms is therefore of great benefit to regulators and the shellfish industry in providing an early warning of toxicity events and also of the decrease in a bloom that will precede the reopening of a closed site. The Marine Research Institute has shown that *A. tamarense* and *A. ostenfeldii* are present at different locations around Icelandic marine habitats.

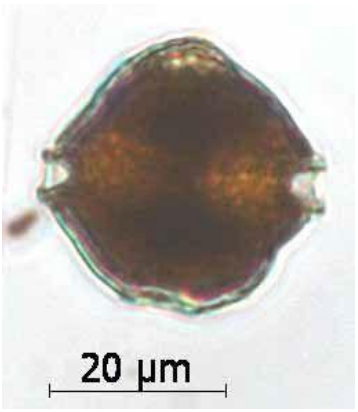


Figure 4: An armoured dinoflagellate belonging to the genus *Alexandrium* (Swan & Davidson 2011).

Among the species of this genus, *A. ostenfeldii* (Paulsen) Balech & Tangen, originally described from northern Iceland as *Goniodoma ostenfeldii* (Paulsen 1904), has been reported from many coastal temperate waters from both hemispheres, but particularly from the north Atlantic (Hansen et al. 1992, Balech 1995). *Alexandrium ostenfeldii* was also identified as the primary, if not unique, source of spirolides, a novel group of macrocyclic imines characterized as marine fast-acting toxins (Cembella et al. 2000). While effects in humans have not been reported yet, in mice, following intraperitoneal injection, these toxins cause similar effects to those caused by DSP toxins, thereby

causing a positive response in the DSP assay. Finally, *A. ostenfeldii* exudates have been shown to be toxic to the tintinnid *Favella ehrenbergii* (Hansen et al. 1992) and certain strains can cause short-term toxic effects towards heterotrophic dinoflagellates (Tillmann & John 2002).

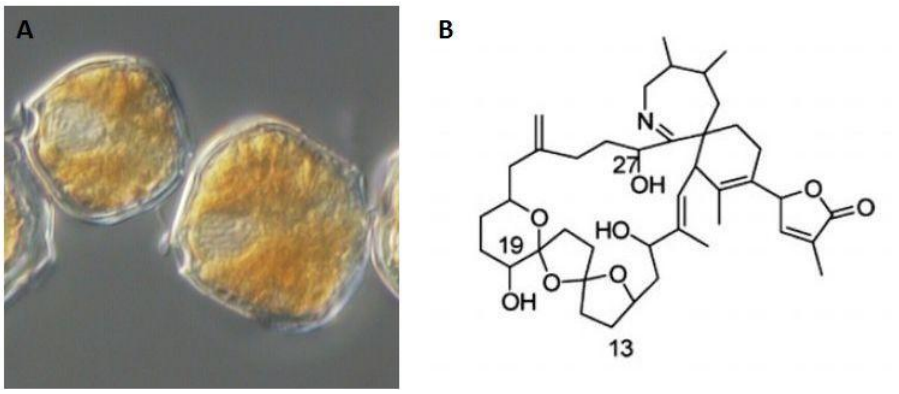


Figure 5: *A. ostenfeldii* (picture: WORMS) Spirolides (SPX): cyclic imines (ca 10 analogues) (Campbell et al. 2011).

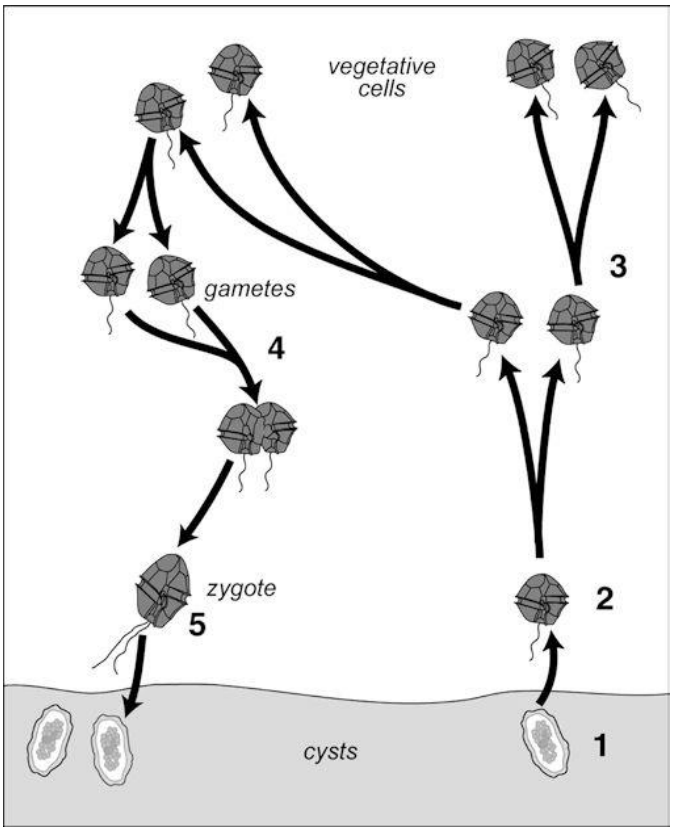


Figure 6: The life cycle of *Alexandrium*, a dinoflagellate with cyst resting stages (1) that can act as reservoirs for new population growth. The resting stages rupture (excyst) to yield swimming cells (2) which continue to divide to produce a vegetative population (3). As nutrients are depleted, division slows and gametes are formed that fuse to form a zygote and then a cyst (4, 5). Adapted from Anderson et al. 1996.

The life histories of most *Alexandrium* species that have been studied involve an alternation between asexual and sexual reproduction (Fig. 8). Repeated divisions (bianry fission) lead to the proliferation

of motile, vegetative cells as blooms develop. This ia an asexual process that terminates when sexuality is induced. Sexuality begins with the formation of gametes which fuse to form swimming zygotes (planozygotes) which in turn become dormant, resting cysts (hypnozygotes; thick-walled). According to Bolch et al. (1991), the term „cyst“will refer to hypnozygotes formed through sexuality. Most species also produce another resting stage called „temporary cyst“ (pellicle= thin-walled) when motile, vegetative cells are exposed to unfavorable conditions such as mechanical shock or a sudden change of temperature or salinity. When conditions become favorable again, temporary cysts quickly re-establish a vegetative, motile existence. The temporary resting state thus allows the cells to withstand short-term environmental fluctuations. The planozygotes that develop after the fusion of gametes swim for up to a week before falling to the sediment as resting cysts to begin dormancy. Here „dormancy“is defined as the suspension of growth by active endogenous inhibition, while „quiescence“as the suspension of growth by unfavorable environmental (i.e. exogenous) conditions. *Alexandrium* cysts typically proceed through a mandatory dormancy period before they are capable of germination (Anderson 1998).

1.3 The Chemical Nature of Phycotoxins

For each of the three main ASP, DSP and PSP toxin groups and subgroups, the occurrence of the toxins, their chemical characteristics, toxicokinetic evaluations, human-exposure assessments and detailed review of potential methods of analysis have in recent years been published by the European Food Safety Authority (EFSA) as scientific opinions (2008-2009). The diversity of the numerous analogues or natural enzymatic metabolites of marine biotoxins has been described (Van Dolah 2000). Figures 7-9 highlight the structure of the parent or reference toxin within each group and an indication of the number of relative analogues or natural enzymatic metabolites. In addition, Table 1 lists the producers of the toxin, mechanism of action and effects in humans, the current European Union (EU) reference methods of analysis and regulatory limits in shellfish meat applied in the monitoring regimes.

1.3.1 Hydrophilic Phycotoxins – The Domoic Acid Group and Saxitoxins

The domoic acid group comprises ten potent water-soluble neurotoxins, domoic acid (DA, Figure 1) and its isomers, which are responsible for amnesic shellfish poisoning (Jeffrey et al. 2004). Domoic acid is a secondary amino acid belonging to the kainic amino acid group. Its chemical structure closely resembles that of kainic acid. Kainic acid was isolated from the seaweed *Digenea simplex* (Wulfen) Agardh and belongs to the same family as *Chondria armata* (Kützing) Okamura (Takemoto, 1978). Both amino acids present neuroexcitatory and neurotoxic activities. Some of domoic acid's structure is similar to that of glutamic acid. And this is why domoic acid behaves as a glutamate receptor agonist, especially in kainic receptors (Meldrum, 1987) of the central nervous system. Continuous activation of this kind of receptors leads to an excessive accumulation of calcium in the cell. Cellular death is the final output. Domoic acid is a small molecule (molecular weight 311.14 Da). Its three carboxylic groups make it highly hydrophilic and polar.

This group of toxins produced by the genus *Pseudo-nitzschia* and *Nitzschia* and *Chondria armata* has a worldwide distribution (Bates et al. 1991, Quilliam 1999, Amzil et al. 2001, Vale 2007). The mechanism of action of these compounds involves their interaction with kainate receptors (KD 5 nM; Van Dolah 2000a), a subclass of glutamate receptors, and their activation. Finally, domoic acid shows limited stability when exposed to factors such as high temperatures, extreme pH, and aqueous

solutions over time. The main decomposition product after high temperature and time exposure is 5'-epi-domoic acid.

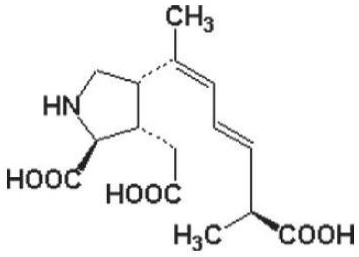
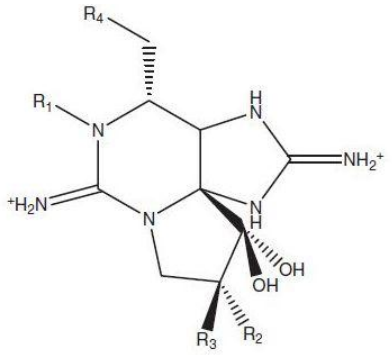


Figure 7: ASP toxins – Domoic Acid (DA; ca 10 analogues) (Campbell et al. 2011).

The second hydrophilic phycotoxin group comprises saxitoxin (STX, Figure 2) and its analogues, more than 24 potent water-soluble neurotoxins that differ in combinations of hydroxyl and sulphate substitutions located at four sites of a tetrahydropurine backbone. Based on substitutions at R4, the saxitoxins can be subdivided into four groups: the carbamate, sufocarbamoyl, decarbamoyl and deoxydecarbamoyl toxins (Van Dolah 2000a). STX and analogues are produced by the genus *Alexandrium, Gymnodinium* and *Pyrodinium* and elicit their effects by binding with high affinity to site 1 of the voltage-dependent sodium channel α-subunit (Noda et al. 1989, Cestele & Catterall 2000) and blocking the sodium influx that prevents the generation and propagation of action potentials in excitable cells (Kao 1966, Caterall 1980).



			Carbamate toxins	*N*-Sulfocarbamoyl toxins	Decarbamoyl (dc) toxins	Deoxydecarbamoyl (do) toxins
R_1	**R_2**	**R_3**	**R_4: $OCONH_2$**	**R_4: $OCONHSO_3^-$**	**R_4: OH**	**R_4: H**
H	H	H	Saxitoxin (STX)	B1 (GTX 5)	dc-STX	do-STX
H	H	OSO_3^-	Gonyautoxin (GTX) 2	C1	dc-GTX 2	do-GTX 2
H	OSO_3^-	H	GTX 3	C2	dc-GTX 3	do-GTX 3
OH	H	H	Neosaxitoxin (NEO)	B2 (GTX 6)	dc-NEO	
OH	H	OSO_3^-	GTX 1	C3	dc-GTX 1	
OH	OSO_3^-	H	GTX 4	C4	dc-GTX 4	

Figure 8: Chemical structure of the parent/reference toxin(s): PSP toxins (<30 analogues) (Campbell et al. 2011).

1.3.2 Lipophilic Phycotoxins – Okadaic Acid, Dinophysistoxins and Others

DSP toxins have in recent times become known as lipophilic toxins incorporating okadaic acid, dinophysistoxins (Vale 2007, Larsen et al. 2007), azaspiracids (Twiner et al. 2008, Furey et al. 2010), pectenotoxins (Halim & Brimble 2006) and yessotoxins (Paz et al. 2008) with the last two not proved to cause diarrheic symptoms following intoxication. These compounds are well known inhibitors of

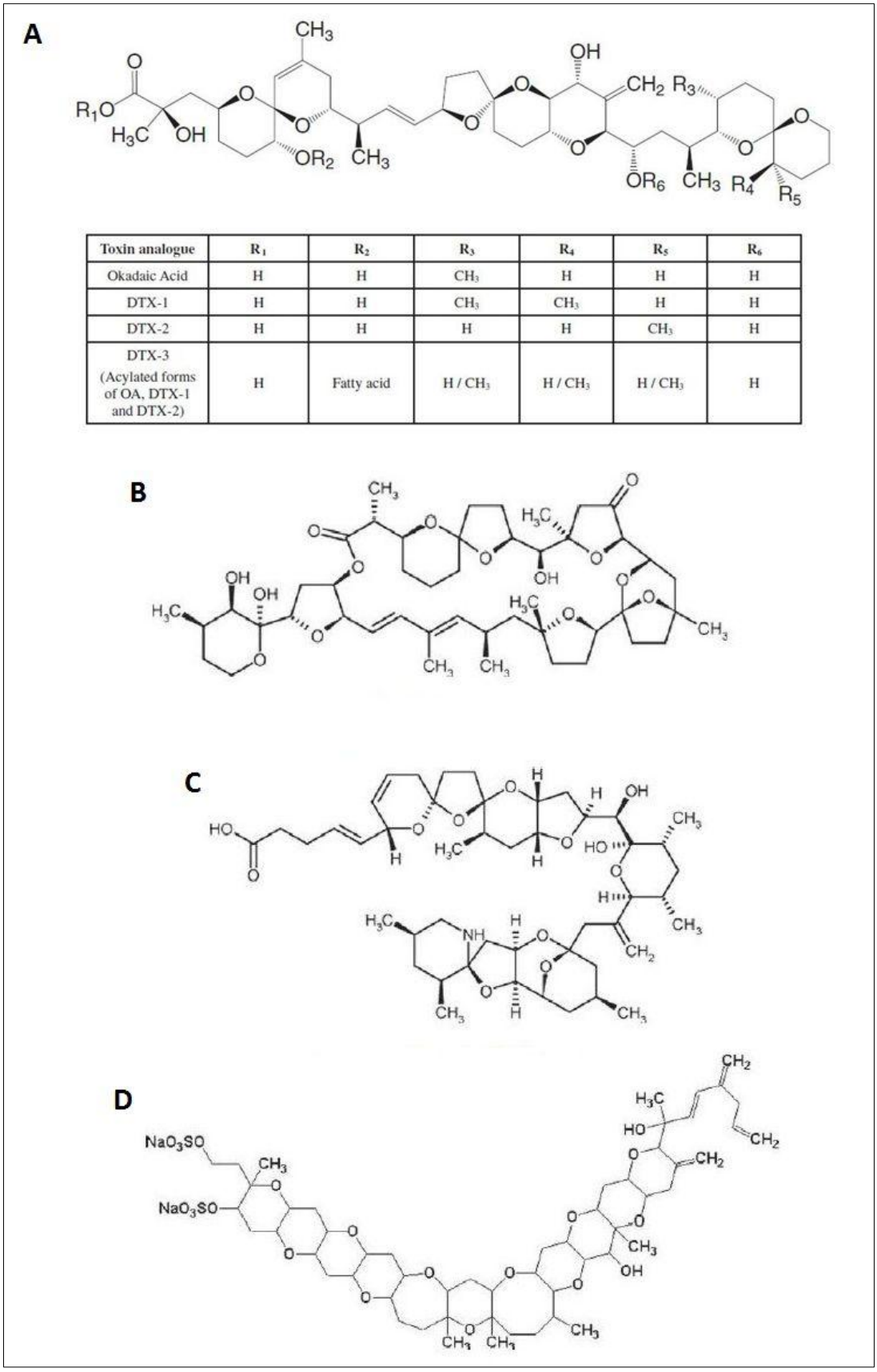
protein phosphatases, mainly PP2A and PP1. The main toxins in the group, okadaic acid and its analogues DTX1 and DTX2, are long chain compounds containing polyether rings and an β-hydroxycarboxyl function, the difference between them being only the number or position of the methyl groups they contain. Their molecular masses are around 810. Some isomers have been found but their precise structure has not yet been elucidated (Quilliam 2003). Several derivatives have also been found both in bivalves and plankton, differing from the original toxins mainly in: a) the esterification of the hydroxyl group in the C7 position with fatty acids of a different chain length but typically C14 to C18, saturated or unsaturated, to produce DTX3 compounds; b) the formation of diol-esters with C7 to C9 unsaturated diols; c) oxidation of the diol part of the molecules of the diol-esters; d) esterification of the diol-esters with sulphated chains, which may or may not include an amide function in the polar side chain, to produce DTX4 and DTX5, respectively; and e) the lack of the hydroxyl group in C2 or C7 (Fig. 9A).

Okadaic acid and its analogues are lipophilic compounds, which are highly soluble in organic solvents such as methanol, acetone, chloroform or dichloromethane. Their derivatives have different polarities and consequently their solubilities in organic solvents are extremely variable. Acyl-derivatives (DTX3) and diol-esters are less polar than their original toxins. On the other hand, oxidised derivatives of diol-esters, DTX4 and DTX5, are more polar than their corresponding toxins and their solubility in some organic solvents such as dichloromethane or hexane is reduced, especially in the case of the two latter types, DTX4 and DTX5, which are water-soluble.

Pectenotoxins are cyclic polyether lactones (Fig 9B), which differ structurally from each other mainly because of: a) the different degrees of oxidation at C18, from methyl to carboxylic acid; b) the arrangement or epimerisation of the spiroketal ring system in rings A and B; and c) the opening of the large lactone ring in C1-C33 (Burgess & Shaw 2001, Quilliam 2003). They are lipophilic and soluble in organic solvents, but some of them, such as aqueous acetonitrile, can produce transformations of the original toxins (Sasaki et al. 1998). They are also easily destroyed under strong alkaline conditions, but detailed stability studies have not been performed.

Azaspiracids are a group of toxins characterised by an unique structure that includes a trispiro ring assembly (Fig 9C), an azaspiro ring fused with a 2-9-dioxabicyclo[3.3.1]nonane and a terminal carboxylic acid group (Daiguji et al. 1998). Their molecular masses are around 840. The differences between them are due the methylation of C8 and C22, and to the hydroxylation of C3 and C23. Azaspiracids are less polar than what would be expected from the presence of an amine and a carboxylic acid, because these two functions appear to form an intramolecular ion pair (Quilliam 2003). They are soluble in organic solvents but unstable in some of them, such as chloroform, under slightly alkaline conditions, and or during chromatography on silicabased supports (James et al. 2000)

Yessotoxins are ladder-shaped cyclic polyethers similar to brevetoxins, with molecular masses of around 1150. Yessotoxin is characterised additionally by the presence of two sulphate esters and a C9 side chain (Fig. 9D). The known analogues differ from yessotoxin in one or two of these characteristics, mainly by desulphation, hydroxylation, carboxylation or by changes in the length of the carbon chain (Satake et al. 1997, Tubaro et al. 1998). Adriatoxin is also a very closely related compound that differs from yessotoxins in that it lacks one ring and the lateral carbon chain (Ciminiello et al. 1998). The presence of sulpho-esters make these molecules more polar than most others in the DSP group, and it is easy to find large amounts of them in the culture medium (enriched or artificial seawater) when the producer dinoflagellates are grown. They can be adequately extracted with aqueous methanol (Yasumoto & Takizawa 1997).



Toxin analogue	R_1	R_2	R_3	R_4	R_5	R_6
Okadaic Acid	H	H	CH_3	H	H	H
DTX-1	H	H	CH_3	CH_3	H	H
DTX-2	H	H	H	H	CH_3	H
DTX-3 (Acylated forms of OA, DTX-1 and DTX-2)	H	Fatty acid	H / CH_3	H / CH_3	H / CH_3	H

Figure 9: Chemical structure of the parent/reference toxin(s): DSP/lipophilic shellfish toxins (LST) A) Okadaic Acid (OA) and Dinophysistoxins (DTXs) (>10 analogues and esters); B) Pectenotoxin (ca. 13 analouges); C) Azaspiracid (ca. 20 analouges) and D) Yessotoxin (ca. 36 analouges) (Campbell et al. 2011).

Furthermore, although okadaic acid (OA), dinophysistoxin-1 (DTX1), dinophysistoxin-2 (DTX2), pectenotoxin-2 (PTX2) and pectenotoxin-11 (PTX11) have been found to be the dominant toxins in Dinophysis species (Fig. 1), it has been shown that esterified okadaic acid analogues such as 7-O-palmitoyldinophysistoxin-1 (dinophysistoxin-3: DTX3) (Suzuki et al. 1999, 2001a) and many of pectenotoxins such as pectenotoxin-6 (PTX6) (Suzuki et al. 1998), pectenotoxin-2 seco-acid (PTX2sa) (Suzuki et al. 2001b,c) are formed by metabolism of parent toxins in shellfish tissues.

1.4 Phycotoxin Detection Methods

As in the case of other marine toxins, two different approaches may be used to address the determination of these toxin groups depending on the type of information required: assay methods and analytical methods. Assay methods measure an integrated biological or biochemical response which is usually converted into equivalents of a representative toxin of the family on the basis of a previous dose/response curve, and this correlates with overall toxicity. Assays for marine toxins comprise *in vivo* bioassays, using live animals, and *in vitro* assays. The latter may be further categorised into functional and structural assays. Functional assays measure a response linked to the action mechanism of the toxin (e.g. phosphatase inhibition assays) and therefore correlate well with real toxicity. Structural assays (e.g. immunoassays) are based on the measurement of the interaction between the antibodies and specific toxin structures that are not necessarily related to the biological activity of the toxin, so, correlation with actual toxicity is not always as good as in the case of functional assays.

Analytical methods generally involve a preliminary toxin separation step and further identification and quantification of the individual toxins by measuring an instrumental response that is proportional to the concentration of the toxin. This requires the previous calibration of the instrumental equipment using toxin standards of each one of the toxins to be quantified. The response should be converted to toxicity values on the basis of specific conversion factors and the overall toxicity is determined as the sum of the individual toxicities. This category of methods includes High Performance Liquid Chromatography (HPLC) with different detection methods (Monochromatic and Spectral UV, Fluorescence, Mass Spectrometry) and Capillary Electrophoresis (see Table 1 for the overview of official methods used in the European Union).

Currently, EU regulations stipulate that the reference methods for the detection of marine biotoxins are two distinct animal bioassays based on the hydrophilic (Hollingworth, & Wekell 1990) and lipophilic (Yasumoto et al. 1978) solvents used for the extraction procedure. This test consists in administration of shellfish extracts to laboratory mice and monitoring the time until death. Three mice have to be used for each test. A sample is considered as positive for the presence of marine toxin when 2 out of 3 mice die within 24 h of inoculation with an extract equivalent to 5 g hepatopancreas or 25 g whole body. The mouse bioassay gives an indication of the overall toxicity of the sample. But, the mouse bioassay has several drawbacks:

- The results show high variability because they depend on strain, gender, sex, state of the health and weight of the animals.
- The mouse bioassay did not show good reproducibility between laboratories.
- It suffers from controversial ethical problems since animals are sacrificed.
- It is expensive due to the animal maintenance.
- The mouse bioassay may give false positives because of interferences by lipids, notably free fatty acids.

- The mouse bioassay shows a low specificity (no differentiation between the various DSP toxins). Even if the mouse bioassay suffers from low specificity, the fact that this bioassay gives an indication about overall toxicity of the sample, can also be considered as an advantage in term of health protection.

Table 1: Predominant toxins covered by European Union legislation, including action, effects and regulatory methods employed.

Toxin group	Reference toxin	Algal genera and species derived from	Action and effects in humans	Current EU regulatory limits (lg/kg of shellfish meat)	Current EU monitoring method	Limit of detection (LOD)/Limit of quantification (LOQ)
PSP toxins	Saxitoxin	**Dinophyceae:** *Alexandrium* spp., *Gymnodinium* spp., *Pyrodinium* spp. **Cyanobacteria:** *Anabaena* spp.; *Aphanizomenon* spp., *Cylindrospermopsis* spp., *Lyngbya* spp., *Planktothrix* spp.	Blockage of site 1 of the voltage-gated sodium channel causing cardiorespiratory failure and death	800 STX Eq	Mouse bioassay with 0.1 M HCL (15 min) HPLC-FLD (Lawrence method)	LOD: 370 lg STX Eq/Kg LOQ: 10–80 lg STX Eq/kg for individual analogues
ASP toxins	Domoic acid	**Bacillariophyceae (diatom):** *Pseudo-nitzschia* spp. **Rhodophyta:** *Chondria armata* (Kützing) Okamura	Interacts with kainite receptors causing neurological damage, memory loss and death	20 000 DA Eq	HPLC-based methods Antibody-based methods(ELISA) (For screening purposes	LOD: 0.2–1 mg DA/kg LOQ: 1–2.5 mg DA/kg LOD: 0.003 mg DA/kg LOQ: 0.01 mg DA/kg
DSP toxins	Okadaic acid and Dinophysistoxins	**Dinophyceae:** *Dinophysis* spp. *Prorocentrum lima* (Ehrenberg) F.Stein	Inhibit protein phosphatases by binding to PP1 and PP2a receptor sites causing diarrhea.	160 OA Eq	Mouse Bioassay or Rat Bioassay with acetone extraction (24 h)	Unknown for each toxin. These bioassays are incapable of detecting these toxins at their current regulatory limit with 100% certainty. For okadaic acid the probability of detection at the regulatory limit is as low as 40%.
	Pectenotoxin-2	*Dinophysis* spp.	In vitro disruption of actin cyto-skeleton and diarrheic effects are in dispute	160 OA Eq		
	Azaspiracid-1	*Azadinium spinosum* Elbrächter & Tillmann	Action is still unknown but causes diarrhea and neurotoxic effects	160 AZA Eq		
	Yessotoxin	*Gonyaulax grindleyi* Reinecke (=*Protoceratium reticulatum*) *Lingulodinium polyedrum* (Stein) Dodge *Gonyaulax spinifera* (Claparède & Lachmann) Diesing	Action not fully known but interacts with phosphodiesterase enzymes and diarrheic effects are being questioned	1000 YTX Eq		

2. Objectives and Aims of the Study

Based on the presented facts and observations we hypothesised that the occurrence of HAB events as well as the species composition of potential toxin producing taxa in Icelandic marine waters is directly related to environmental features of the habitat (e.g. seasonal variations in temperature, changes in macronutrient compositions due to communal and aquacultural effluents) and that the amount of ASP, PSP and DSP toxins as well as the chemical consistencies of such toxins varies with the combination of different abiotic characteristics in the environment. Moreover, the requirement of a valid monitoring system for the HAB predictability is one of the most important factors for future projectable investments of the Icelandic mussel industry. Thus, we incorporated in our monitoring different methods which are used by official European environmental agencies and/or newly developed ones suitable for the Icelandic setting, including for example the tests of solid-phase adsorption toxin tracking (SPATT) bags. Finally, in order to replace the difficult and time-consuming counting method according to Utermöhl and as a useful instrument for future monitoring, the development of a real-time polymerase chain reaction (PCR) method for a fast screening of phytoplankton samples on incidences of PSP toxin producing *Alexandrium tamarense* species was one main objective of the present study.

Therefore, the aims of the present study were:

1. to monitor mussels and phytoplankton samples at two different stations in the north-west of Iceland for the presence of potentially toxin producing algal taxa and their toxins in relation to environmental characteristics such as temperature and salinity over a two year period during the main seasons (spring, summer, autumn);

2. to find relationships in the occurrence of toxin producing taxa and the composition of macronutrients such as nitrate, ammonium, nitrite, phosphate and silicate in the environment;

3. to test the suitability of adsorption agents in the conglomeration of ASP,PSP and DSP toxins from the water column for their potential use to monitor future toxicity events;

4. to gain information about the chemical nature of the ASP, PSP and DSP toxins attendant in the monitored environment by LC-MS analysis;

5. to test the reliability of rapid toxin tests such as the Jellett® test, the Biosense® Laboratories ASP test, the DSP OkaTest® (ZEU-IMMUNOTEC) and the Ridascreen® Fast PSP test, in respect to their accordance to LC-MS measurements in plankton and mussel samples;

6. to isolate and grow toxin and non-toxin producing *A. tamarense* species, using commercial strains and isolated individuals from the two stations, in order to gain DNA material for the qPCR method development and to confirm the functionality of the developed test;

7. to test environmental samples for the presence of PSP toxin producing *A. tamarense* species.

3. Material and Methods

3.1 Study Sites

The two sampling sites were located in the north-western part of Iceland (Fig. 10A) and were chosen according to distinct differences in their environmental conditions. The first site is Miðfjörður nearby the town of Hvammstangi (UB; 65°23,62´N; 20°59,91´W), which is a long and narrow fjord. The second site lies outside of Höfði at Skagaströnd (ST; 65°49,56´N; 20°19,58´W) and faces the open ocean (Fig. 10B). While at the former one experimental mussel farming is ongoing with the hope it will develop into a profitable local industry in the future, the latter one is not part of such programs yet.

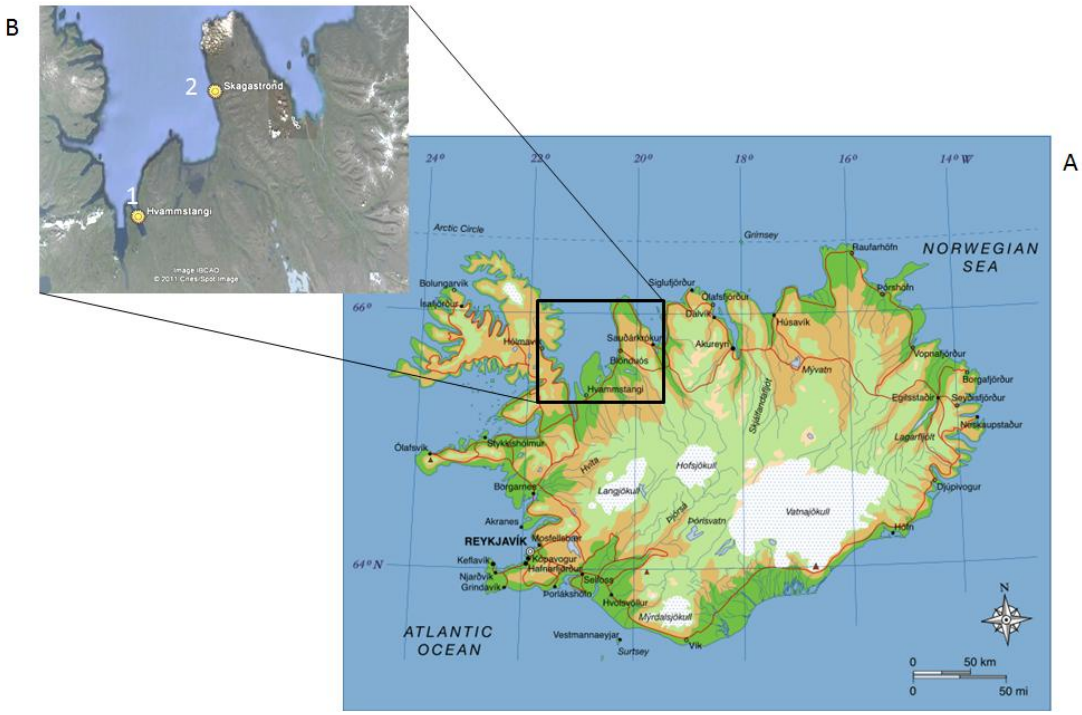


Figure 10. Sampling locations in the north-western part of Iceland. A) Survey map; B) Sampling sites (1. Miðfjörður, 2. Skagaströnd)

3.2 Sampling Events and *in situ* Measurements

Overall, 78 samples were collected the monitoring events in 2011/2012. 34 samples were taken in 2011 (19 at Miðfjörður; 15 at Skagaströnd), whereas 44 samples were collected in 2012 (23 at Miðfjörður; 21 at Skagaströnd). The sampling for the project started officially in March 2011 at Miðfjörður, whereas the weekly sample collections followed from May onwards until September. In 2012, the sampling was conducted from April 17th at Miðfjörður and April 26th at Skagaströnd to September 28th at Miðfjörður and October 4th at Skagaströnd, respectively. Weekly campaigns were started in May ongoing to September in 2012 (see Table A, in the appendix for the individual sampling dates). During the sampling events salinity and temperature were measured *in situ*, using handheld probes (YSI Professional Plus Series, YIE 100 993). Since salinity is a ratio, the value is actually dimensionless (no units) and will be therefore referred in the further as practical salinity units (PSU). In addition to the salinity, the depth of the euphotic zone was measured with a Secchi

disk. In order to reflect the range of depth most commonly used in mussel farming, the data of the *in situ* measurements and all samples were obtained from 3, 10, and 15 m depths at each location.

3.3 Sample Taking and First Processing

3.3.1 Water Samples

Samples from the different depths for phytoplankton enumeration as well as the chlorophyll a and macronutrient analysis were collected using a Niskin water column sampling device (KC-Denmark, Fig. 11A). For the phytoplankton analysis approximately 200 mL of each sample was placed in an opaque glass bottle, mixed with 2 mL Lugol´s solution to preserve the sample. From the water samples 700 mL were placed into 1 L bottles for the determination of macronutrients and chlorophyll *a*, respectively, without a first filtering. All samples were stored at 4 °C in the dark.

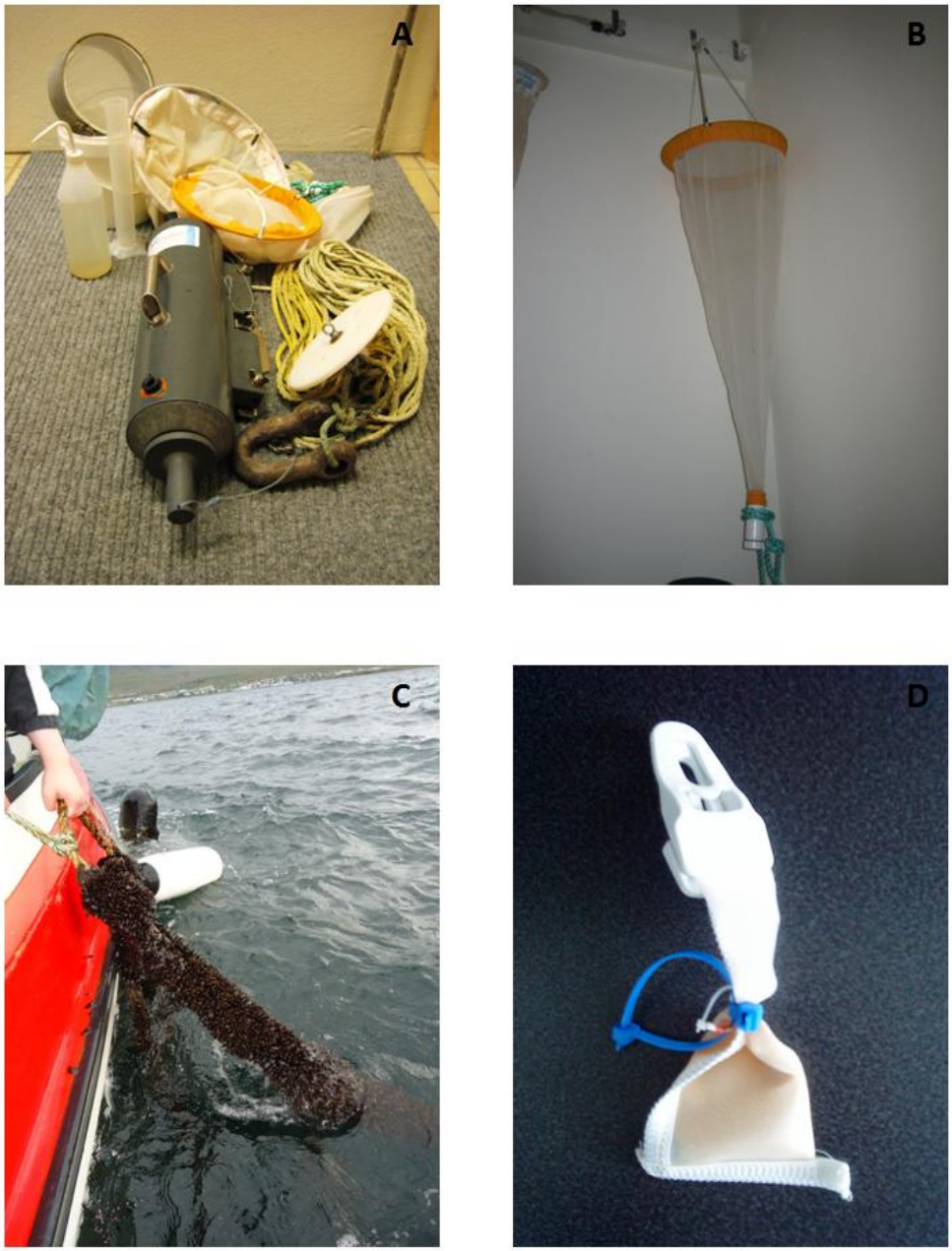


Figure 11: Sampling equipment (A) Niskin sampler, B) Plankton net, C) Mussel cages) and D) SPATT bag).

Additional water samples were taken in Miðfjörður and near Skagaströnd for the real-time PCR method development during the monitoring in 2012, using a 12 m hose allowing an integrated water sample to be collected. The end of a hose was immersed down to 10 m depth and the end of the hose was closed with a cork. Then the hose was pulled on board. The cork was taken from the end of the hose and the seawater was collected in 3 x 5 L container. The procedure was repeated until the required volume was filled. In the laboratory, samples were subdivided into two parts and filtered (5 µm pore size, nylon mesh, Normesh Limited, Manchester, UK). Filters were diluted with 5 mL phosphate buffered saline (PBS, 137 mmol/L NaCl, 2.7 mmol/L KCl, 10.0 mmol/L Na_2HPO_4 x 2 H_2O, 2.0 mmol/L KH_2PO_4, pH 7.4) and vigorously vortexed to remove the organic material from the filters. Then filers were removed and the suspensions centrifuged at 5500 rpm for 20 min under cooling conditions (4 °C). The supernatant was decanted and the first part of the remaining cells was preserved in 1 mL pre-chilled methanol (HPLC grade, Sigma-Aldrich), while the second one was fixed in 1 mL RNA*later*™ (Sigma-Aldrich). Samples were finally stored at -80 °C until further processing.

3.3.2 Phytoplankton-net Samples

A phytoplankton net (mesh size of 15 µm, Fig. 11B) was lowered to a depth of 10 meters and then pulled up slowly to the surface (ca. 0.5 m/s). The collection chamber was emptied into an opaque glass bottle (500 mL volume); 1 mL of Lugol´s solution was added and the samples stored at 4 °C.

3.3.3 Mussel Samples

Mussels for the toxin analysis were kept throughout the sampling periods in special constructed tiered cages at both sampling locations. The cages were cylindrical (150 cm length and 40 cm wide) and made of mesh-covered aluminium. 40-60 g of mussels was collected from the cages on every sampling date (Fig. 11C). Tissues were removed from the shell, transferred to strainers, and drained for 5 min before homogenization (blender). The homogenate (at least 50 g) was then distributed into plastic containers (10.0 ± 0.1 g), frozen, and stored at –20 ± 2°C until analysis.

3.3.4 Solid-phase Adsorption Toxin Tracking (SPATT) bags (2011)

This technique is based on the passive adsorption of biotoxins onto porous synthetic resin filled sachets (SPATT bags, Fig. 11D). The SPATT bags were made from 95 µm nylon mesh, sewn with polyester thread (dimensions: 60×60 mm) and were filled with suspended SEPABEADS SP700 as adsorption agents. Into one seam a length of polyester string was sewn to fix the bags to the submerged mooring frame of the mussel cages (3-5 m depths). Care was taken that the bags did not dry out before being placed in the water. After the retrieval of the bags from the mooring line they were placed on ice for transport to the laboratory where they were stored frozen (-20 ^{o}C) prior to extraction and analysis. The bags were retrieved and replaced at each sampling event during the monitoring in 2011 at both stations.

3.4 Laboratory Analysis of Environmental Parameters

3.4.1 Macronutrients (2011)

The water samples were filtered using a 47 mm Whatman GF/F microfilter and stored at 4 °C. The macronutrients from 2011, ammonium, phosphate, silicate, nitrate and POCN (total N and C), were analysed by our Scottish cooperation partner according to the methods given in Davidson et

al. (2007). The nutrient ratios were calculated according to Redfield et al. (1963) and Brzezinski (1985).

3.4.2 Chlorophyll *a* (chl *a*, 2011/2012)

The water samples were filtered using a 47 mm Whatman GF/F microfilter under low light conditions. The filters were stored at -80 °C. A spectrophotometric method adapted from Lorenzen (1967) was used to ascertain chl *a* content in the samples. A correction of the chl a data for phaeophytin content (degradation product, not analysed) was not conducted.

3.4.3 Cell Counts (2011/2012)

While the plankton net samples were only used for the notification of accompanying phytoplankton taxa, the Niskin water samples were used for the quantification of the potentially toxin producing microalgae on genus level (*Alexandrium* spp., *Dinophysis* spp., *Pseudo-nitzschia* spp.). In general, the plankton net sample was viewed before the quantification samples. Microscopic analysis was conducted using an inverted light microscope (Olympus IX-51) in combination with counting chambers according to the method of Utermöhl (1958); using Hydro-Bios settling chambers. Overview samples were placed in 10 mL settling chambers, whereas the quantification samples settled in 50 mL chambers. The samples were allowed to settle overnight (17-24 hours), following the sampling protocol described by the "National Reference Laboratory (NRL) Standard Operating Procedure" for the collection and analysis of water samples for toxic phytoplankton species which is used at the Scottish Association of Marine Science (SAMS, UK). Final cell densities were calculated according to the following formula (low and high density analysis):

Low density analysis: For each individual target genus, the concentration of cells per litre is calculated as:

$$\text{number of cells observed} \quad X \quad \left\{ \frac{1000}{\text{volume settled [mL]}} \right\}$$

High density analysis: For each individual target genus, the concentration of cells per litre is calculated as:

$$\left\{ \frac{\text{total numbers of cells counted}}{\text{number of FOV}} \right\} \quad X \quad (\text{MFF}) \quad X \quad \left\{ \frac{1000}{\text{Volume settled [mL]}} \right\}$$

Abbreviations: FOV: fields of view, MFF: microscope field factor

For the high density analysis, the cells of target organisms were counted in a minimum of 10 randomly selected fields of view (FOV). The number of FOV was determined to ensure that a minimum of 100 cells of the target species were counted. The microscope field factor (MFF) was calculated using the formula of the base plate area ($\pi \bullet r^2$, with π=3.1416 and r^2=radius of the circle, squared).

3.5 Toxin Analysis

Nine different methods were applied for the toxin analysis of the mussel samples in 2011/2012 and the SPATT bags in 2011 (Table 2).

Table 2. Survey of the conducted toxin tests in 2011 and 2012, including informations about method and sensitivity.

Toxin group / regulatory limits	Test method	Sensitivity	Supplier
ASP (20 mg domoic acid/kg)	Antibody based lateral-flow test	qualitative	Jellett® ASP Rapid Test
	Antibody based ELISA test	semi-quantitative	Biosense® Laboratories, Norway, ASP ELISA
	LC-MS/MS system (Model: 3200 QTRAP from AB SCIEX)	fully quantitative	Marine Scotland (MARLAB), Aberdeen, UK
DSP (160 µg of okadaic acid equivalents/kg)	Antibody based lateral-flow test	qualitative	Jellett® DSP Rapid Test
	Functional assay: Phoshatase inhibition assay (PP2A = protein phosphatase 2 assay)	Quantitative (sum of OA, and dinophysis toxins 1,2,3)	OkaTest®, ZEU Immunotec
	LC-MS system (Model: API 150 EX from PE SCIEX)	fully quantitative	Marine Scotland (MARLAB), Aberdeen, UK
PSP (800 µg of saxitoxin equivalents/kg)	Antibody based lateral-flow test	qualitative	Jellett® PSP Rapid Test
	Antibody based ELISA test	semi-quantitative	R-BioPharm (Ridascreen®I
	HPLC system (Model: Prominence from Shimadzu), using an FLD (Jasco FP-2020)	fully quantitative	Marine Scotland (MARLAB), Aberdeen, UK

3.5.1 Extraction Procedures for SPATTs and Mussel Samples

3.5.1.1 ASP Toxin Group

SPATTs:

Prior to analysis, the SPATT bags were defrosted at room temperature for 1 hour. The resin (SP-700) was transferred from the mesh bag to a Duran bottle using a funnel, by turning the mesh bag inside out over the funnel. Deionised water was used to rinse off the resin from the bag and the funnel. The volume was made up to approximately 200 mL, using deionised water. Then the bottle was closed with a screw cap and shake by hand for 1 minute (± 15 seconds). On a manifold were two reservoirs installed (25 mL) each pre-fitted with a frit (20 µm) and the resin slurry was poured evenly in both reservoirs. Afterwards, the resin contained in both reservoirs was rinsed with distilled water (100 ± 10 mL). Once the resin in the reservoir has been washed and the water removed after application of a small vacuum, 10 mL of a 1:1 methanol-water solution was added. The reservoir was capped at the top and bottom (below the tap from the vacuum manifold). The contents of the cartridge were mixed using a vortex mixer (ca. 1 minute) and the contents were allowed to soak in methanol (30 ± 5 min) prior to the collection of the eluent. A 100 mL Duran bottle was placed in the manifold directly under the cartridge and the tap was opened. The first 10 mL of extract were collected. Once finished, the extract was mixed by hand for 10 seconds. Then the ASP extract was ready for analysis by ELISA.

Mussel samples:

4 g of mussel homogenates were weighted into a 50 mL centrifuge tube and 16 mL of Extraction solution (50% methanol in dest. water) were added. The solution was mixed well by vigorous shaking on a vortex for 1 min and then centrifuged at 3000 x g for 10 minutes at room temperature. The supernatant was retained for further dilution prior to analysis. The extracts were freshly used for analysis.

3.5.1.2 DSP Toxin Group

SPATTs:

Once the resin in the reservoir has been washed and the water removed after application of a small vacuum, 10 mL of methanol (MeOH) was added. The reservoir was capped at the top and bottom (below the tap from the vacuum manifold). The contents of the cartridge was mixed using a vortex mixer (ca. 1 min) and the contents were allowed to soak in methanol (30 ± 5 min) prior to the collection of the eluent. After this, a 100 mL Duran bottle was placed in the manifold directly under the cartridge and the tap was opened to collect the first 10 mL of extract. From this first extract, an aliquot of 1.5 mL was taken and transferred into a 2 mL vial. This aliquot was then be used for the PP2A analysis. 90 mL of methanol were poured in the reservoir (roughly 10 mL at a time) and the extract thereby slowly eluted and then mixed for 10 seconds. The DSP extract (1.5 mL) is ready for PP2A analysis. The SPATT extract (as described in the PP2A manual) was not hydrolysed as the presence of esters of OA/DTXs was due to the recommendation of the MARLAB highly unlikely.

Mussel samples:

The stored portions (5 ± 0.1 g) were extracted by adding 25 mL of methanol (100% v/v) and mixing with a vortex for 2 min. The methanolic extract was separated by centrifugation for 10 min. at 2000 × g. To perform the hydrolysis, 640 µL of the methanolic extract and 100 µL of 3 N NaOH were mixed and incubated for 40 ± 1 min. at 76 ± 1 °C. To stop the reaction, 80 µL of HCl were added and sample preparation buffer used to make up a final volume of 20 mL.

3.5.1.3 PSP Toxin Group

SPATTs:

Were not analysed due to the inability of the used resin (SP700) to adsorb this particular toxin group.

Mussel samples:

10 g of homogenized mussels were mixed with 10 ml HCl (0.1 M) and boiled for 5 min at 95 °C. After this, samples were centrifuged for 10 min at 3500 x g under cooling conditions (4 °C). The pH-value was controlled after centrifugation adjusted with 5 N HCl when over 4. 100 µL of the supernatant was used and filled up to 1 ml (1:10 dilution) with sample dilution buffer (manufacturer).

3.5.2 Commercial Test Kits – Test Principles and procedures

3.5.2.1 Jellett® Rapid Testing Ltd.

Assay principle

The test uses polyclonal PSP toxin antibodies and works on the principle of lateral flow immuno chromatography using a strip format (similar to pregnancy kits, Fig. 12). The schematic in Figure illustrates positive and negative tests. The test is claimed to detect PSP toxins above 40 µg/100g shellfish tissue in 35 minutes (manufacturer's website).

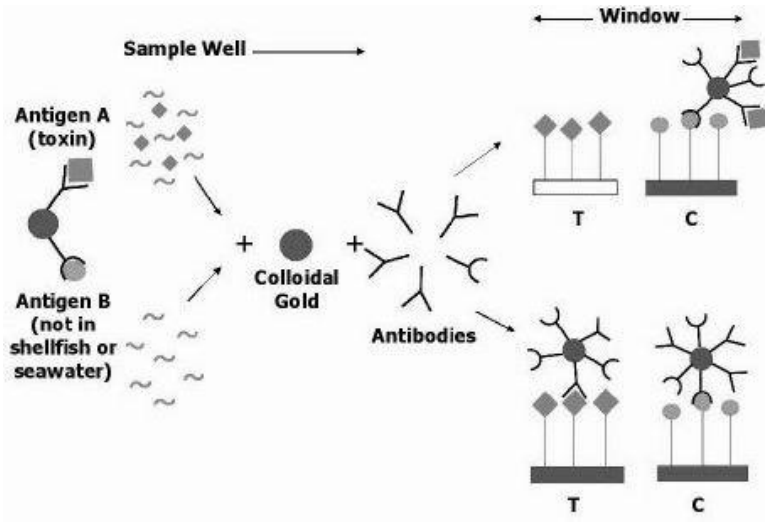


Figure 12. Schematic illustration of the lateral flow immune chromatography test principle for marine biotoxins.

Assay procedure:

Analysis involves dilution of a shellfish extract in JRT running buffer, loading the sample on to a JRT detection strip and waiting for 35 minutes for a colour reaction to develop. At that time, following the manufacturer's instructions, the colour intensity of a test line (or T line) is read against that of a control line (or C line) to define whether the sample is positive or negative. 400 µl of JRT running buffer was placed into individual microcentrifuge tubes using a calibrated pipette and 100 µl of test sample added and mixed by pipetting. 100 µl of the mixture was then placed onto the sample pad of the JRT detection strip. JRT strips were incubated at room temperature for 35 minutes prior to reading. Results were interpreted according to the JRT instruction sheet supplied with the batch of kit.

3.5.2.2 ASP (Biosense® Laboratories, Norway)

Assay principle

Enzyme Linked Immunosorbent Assay (ELISA) has proved to be a sensitive and rapid method for detection of DA in the marine environment (Garthwaite *et al*. 2001). This quantitative DA ELISA was developed by AgResearch (Hamilton, New Zealand) for the detection of DA in water samples, shellfish and algal extracts, and is based on antibodies described by Garthwaite *et al*. (1998).

The ASP ELISA assay is in a direct competition format, where free DA in the sample competes with DA-conjugated protein coated on plastic wells for binding to anti-DA antibodies free in the solution (Fig. 13). The polyclonal ovine anti-DA antibodies are conjugated to horseradish peroxidase (HRP). Sample diluted in buffer is incubated in the wells with the anti-DA-antibody-HRP conjugate. After washing, the amount of conjugate remaining bound to the well is measured by incubation with a substrate that gives a blue product upon reaction with the HRP enzyme. Addition of acid stops the reaction and changes the product colour from blue to yellow. The colour intensity is measured spectrophotometrically on a plate-reader at 450 nm, and is inversely proportional to the concentration of DA in the sample solution. The assay is calibrated using dilutions of a DA calibration solution supplied with the kit. The calibrated range of the assay (I20 - I80) is approximately 10 to 300 pg/mL of DA.

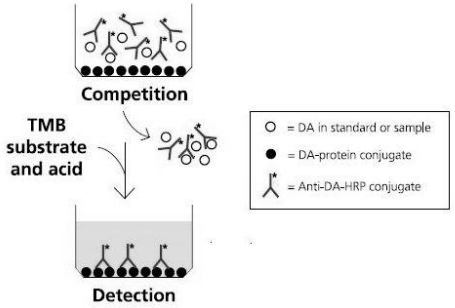


Figure 13. Schematic illustration of the competitive ASP ELISA.

Assay procedure:

Before starting the assay buffers and reagents as well as the ten standards (10-point calibration) were prepared. The first step comprises the incubation of standards and samples with antibody. For this purpose pre-coated plate strips were equilibrated with 300 µL Washing buffer (PBS-T; 0.05% Tween 20 in PBS) and the wells were pre-soaked for 5-10 minutes. After removal of the washing buffer 50 µL Standard/Sample buffer (10% methanol in PBS-T) were added to each of the duplicate Amax and Blank wells. Then 50 µL Antibody-HRP ovalbumin buffer (1% ovalbumin) were added to the Blank wells and 50 µL of each DA standard and sample dilution to each of two wells. Finally, 2.5 ml from vial E (concentrated Anti-DA-HRP) were transferred to a Falcon type tube containing 5.0 mL (for 8 strip assay) Antibody-HRP ovalbumin buffer (prepared vial F) and vortexed briefly. 50 µL of the diluted Anti-DA-HRP conjugate were added to all wells except the Blank wells. The strips were sealed with a plate sealer and incubate at room temperature (20-25°C) for 1 hour. After the incubation step all the contents were removed by inverting the strips over a sink and tapped to remove remaining liquid. The wells were washed 4 times with 300 µL Washing buffer per well. 100 µL of TMB peroxidase substrate were added to all wells and incubated at room temperature (20-25°C) for 15 minutes. During all steps plates were protected from light. The reaction was stoped by adding 100 µL 0.3 M H_2SO_4 to all wells. After 2-5 minutes, the the absorbance was readed in a microplate spectrophotometer using a 450 nm filter.

3.5.2.3 DSP (OkaTest®, formerly Toxiline-DSP, ZEU INMUNOTEC)

Assay principle

OkaTest is an enzymatic test based on a colorimetric PPIA for quantitative determination of OA and other toxins of the OA group, including DTX1 (35-methylokadiac acid), DTX2, and their ester forms. The toxicity of the OA toxins group is directly related to its inhibitory activity against a family of structurally related protein phosphatases (PPs), in particular PP1 and PP2A. PP1 and PP2A are the two predominant forms of protein ser/thr phosphatases found in most mammalian cells (Cohen & Cohen et al. 1989, Cohen et al. 1988). The PP2A heterotrimeric protein phosphatase, is a ubiquitous and conserved serine/threonine phosphatase with broad substrate specificity and diverse cellular functions. Among the targets of PP2A are proteins of oncogenic signaling cascades, such as proto-oncogene serine/threonine-protein kinase. The PP2A family of enzymes represents a major class of serine–threonine PPases, which have been implicated in the regulation of many cellular events. OkaTest uses this strong inhibitory activity to determine the OA content in shellfish using the PP2A with a chromogenic substrate for this enzyme (Fig. 14). After the substrate's hydrolysis by the enzyme, the product can be measured at 405 nm by a microplate reader. As the ability of the PPs to hydrolyze the substrate depends on the amount of OA and analogs in the samples, the toxin concentration can be calculated by using a standard curve. Total content of OA group toxins is determined after an alkaline hydrolysis of the methanolic extract which converts any acylated esters of OA and/or DTXs to their parent OA and/or DTX toxins.

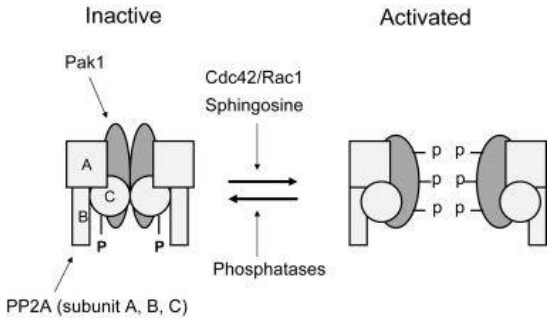


Figure 14. Schematic illustration of the activated and inactivated PP2A enzyme.

Assay procedure:

This kit includes a 96-well microtiter plate, four vials of lyophilized protein phosphatase 2A (PP2A), purified from human red blood cells, five OA standards (0.5, 0.8, 1.2, 1.8 and 2.8 nM) prepared from the OA Certified Reference Material (NRC CRM-OA-c, NRC-CNRC, Institute for Marine Biosciences), a liquid chromogenic substrate (p-nitrophenyl phosphate), phosphatase dilution buffer and buffer solution. The assay procedure and calculations followed the instructions given in Smienk et al. (2012). In detail, the lyophilized phosphatase (PP2A) was rehydrated by adding 2.0 mL phosphatase dilution buffer to the vial and mix gently for 60 ± 5 min at room temperature (22 ± 2°C) by hand. 50 µL each sample extract or standard were added to the wells in duplicate. 70 µL phosphatase solution was added to each well. Then the plate was covered with the adhesive film provided in the kit, mixed by gentle tapping on the side and incubated at 30 ± 2°C for 20 ± 0.5 min. After removing of the adhesive film and 90 µL chromogenic substrate were added to each well and mixed again by tapping gently on the side.

The final incubation at 30 ± 2°C for 30 ± 0.5 min followed the reading of the absorbance at 405 ± 10 nm.

3.5.2.4 PSP (Ridascreen® Fast PSP)

Test principle

The basis of the test is the antigen-antibody reaction, a competitive ELISA for the quantitative analysis of saxitoxin and related toxins, based on antisaxitoxin antibodies that bind PSP toxins with different affinities: saxitoxin 100%, gonyautoxins 2, 3 70%, decarbamoyl saxitoxin 20%, and neo-saxitoxin 12%. The microtiter wells are coated with capture antibodies directed against anti-PSP antibodies. PSP standards or sample solutions, PSP enzyme conjugate and anti-PSP antibodies are added. Free PSP and PSP enzyme conjugate compete for the PSP antibody binding sites (competitive enzyme immunoassay). At the same time, the anti-PSP antibodies are also bound by the immobilized capture antibodies (Fig. 15). Any unbound enzyme conjugate is then removed in a washing step. Substrate/chromogen is added to the wells, bound enzyme conjugate converts the chromogen into a blue product. The addition of the stop solution leads to a colour change from blue to yellow. The measurement is made photometrically at 450 nm. The absorbance is inversely proportional to the PSP concentration in the sample.

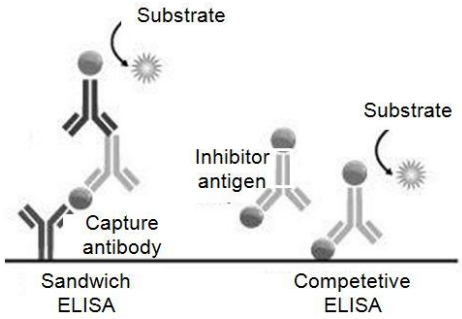


Figure 15. Schematic illustration of the sandwich and competitive PSP ELISA.

Assay procedure:

The ELISA was carried out according to the kit user's manual. Briefly, 50 µL of six saxitoxin standard solutions with concentrations ranging from 0 to 40 ppb (0, 2.5, 5, 10, 20, and 40 ppb) or diluted shellfish extracts (range 1:40 to 1:640, depending on the toxin concentration of each sample) were added into separate wells. The same volume of diluted enzyme conjugate and anti-saxitoxin antibodies in solution was added into each well. The plate was mixed and incubated for 15 min at room temperature. After washing three times with deionised water, 100 µL of substrate cromogen was added to each well and incubated for 15 min in the dark. Colour reaction was stopped with 100 µL of H_2SO_4 0.5 M and the absorbance at 450 nm was measured. All standards and samples were tested in duplicate. A calibration curve was constructed using six standards concentrations (0 to 40 ppb). The working range (where the curve was linear showing a regression coefficient >0.97) of the calibration curve, usually ranging from 0 to 20 µg saxitoxin equivalent per kg shellfish meat, was used to calculate the toxin concentration.

3.5.3 Liquidchormatogaraphy (LC, 2011-2013)

Additionally selected SPATT and mussels samples were analysed by the MARLAB in Aberdeen, Scotland, UK. Liquid chromatography (LC) coupled with mass spectrometry (MS) was used to search for the following lipophilic toxins (LSTs): okadaic acid (OA), dinophysistoxins (DTXs), pectentotoxins (PTXs), azaspiracids (AZAs), yessotoxins (YTXs), spirolides (SPXs). In particular, for PSP (STX = Saxitoxin) the HPLC-FLD method was used, whereas ASP, DSP (hydrolysed and non-hydrolysed) and SPX were analysed using LC-MS/MS. The ASP and SPX methods have been developed in Scotland, UK and are not yet been published, whereas LSTs and spirolides were analysed according to the method of van de Riet et al. (2009).

3.6 Isolation and Cultivation of *Alexandrium tamarense* (Lebour) Balech (2013)

For the isolation of *Alexandrium tamarense* from resting spores, sediment samples were collected from different sites in March 2013. Five gram (wet weight) aliquots of the sediment samples were suspended in autoclaved filtered seawater and sieved to obtain the size fraction between 20 and 150 µm. The material remaining on the 20 µm netting was then washed with autoclaved filtered seawater into a 10 ml glass centrifuge tube, and the resulting suspension was used for the resting cyst isolation. After germination, the isolated cells were grown in SWES medium according to the receipt given at www.epsag.uni-goettingen.de, enriched with Tropic Marin®, Aquarientechnik, Wartenberg, Germany). In addition to the isolation of cells, two strains were bought from the culture collection of algae and protozoa from the Scottish Marine Institute (SAMS, UK) one non-toxic strain (CCAP 1119/9) and a toxic one (CCAP 1119/17). Both strains should be used as control for the development of a real-time PCR method and finally, in order to confirm the production of PSP toxins in *A. tamarense* isolates. These strains as well as the isolates were attempted to grow at 18-22°C in L1 medium according to Guillard & Hargraves (1993).

3.7 Statistical Analysis

With the exception of the chl a concentrations from 2012, all data in the present study were only analysed and measured once. Therefore mean values do not exist and statistical analysis has to be considered with caution. To assess temporal and spatial variability of biomass (chl *a*), algal abundances, and environmental variables, analyses of variance (ANOVA) were performed. Data was logarithmically transformed data, to achieve normality. Relationships between the toxin production and physico-chemical characteristics were evaluated using Spearman rank correlation (r_s) to avoid problems associated with non-normal data distribution. The sensitivity of toxin tests were analysed by regression analysis of cELISA/PP2A test results against LC data. Canonical Correspondence Analysis (CCA), with a forward selection of variables, was then applied to evaluate the relationship between the abiotic variables and the genera composition as well as the toxicity. This analysis was conducted done using a covariance matrix with logarithmic transformation [log (x + 1)] for the environmental data and variation range for the biological data. Altogether, different environmental variables (in 2011: temperature, day length, salinity, PO_4, NH_4, NO_4/NO_2, SiO_3; in 2012: temperature, day length, salinity, Secchi depths, chl *a*) and 9 biological variables (cell counts and toxin concentrations) were used for each station in 3 m depth. In order to verify the probability that the eigenvalues of the axes had been attributed by chance, the Monte Carlo Test was applied (999 interactions; $P \leq 0.05$). All tests (incl. post-hoc) were performed with the program XLSTAT 2011, Version 2011.2.08 Addinsoft.

4. Results

4.1 Abiotic Parameters – *In Ssitu* Measurements and Macronutrient Concentrations

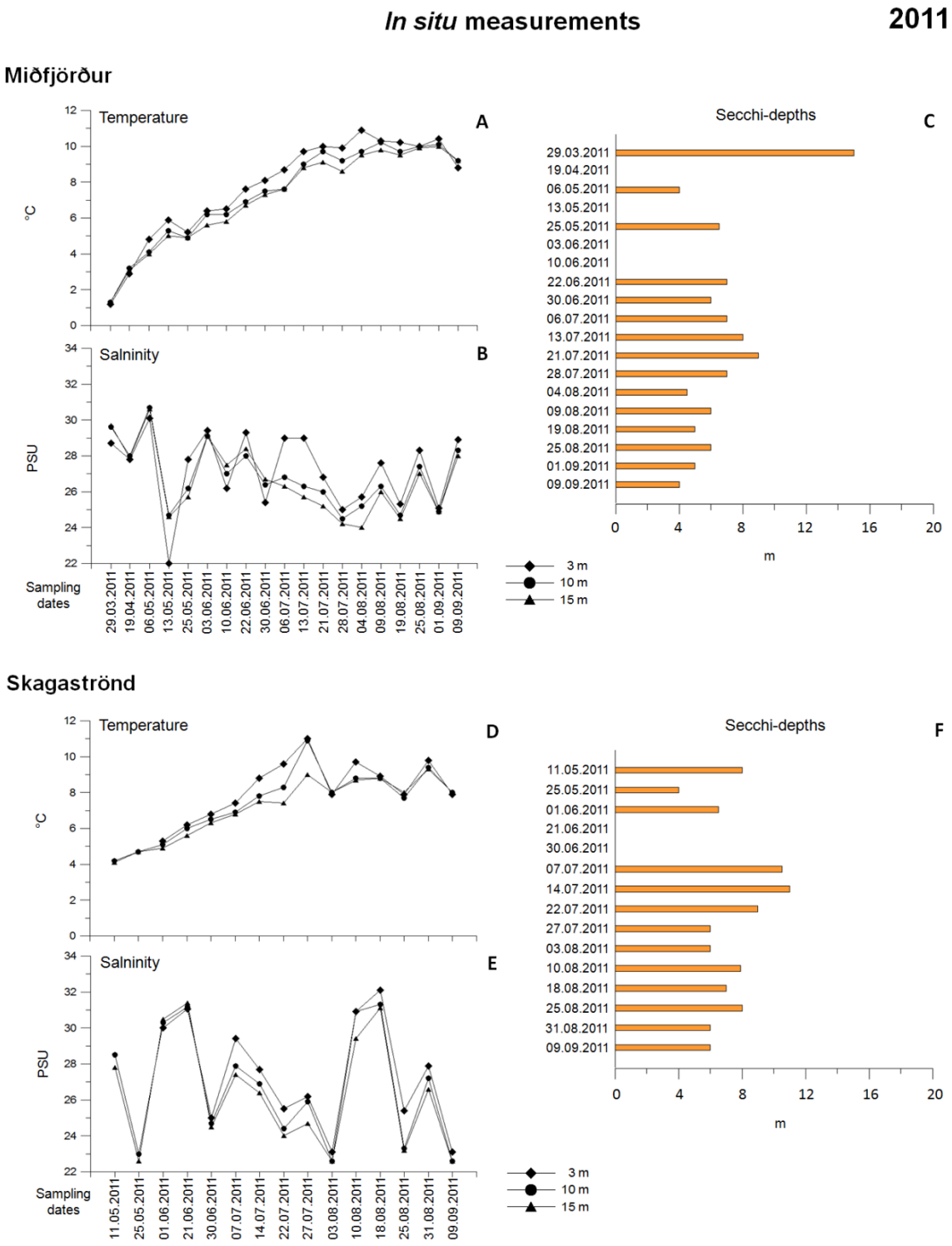


Figure16. Results of the *in situ* measurements (A, D: temperature, B, E: salinity, C, F: Secchi depths) at Miðfjörður and Skagaströnd in 2011.

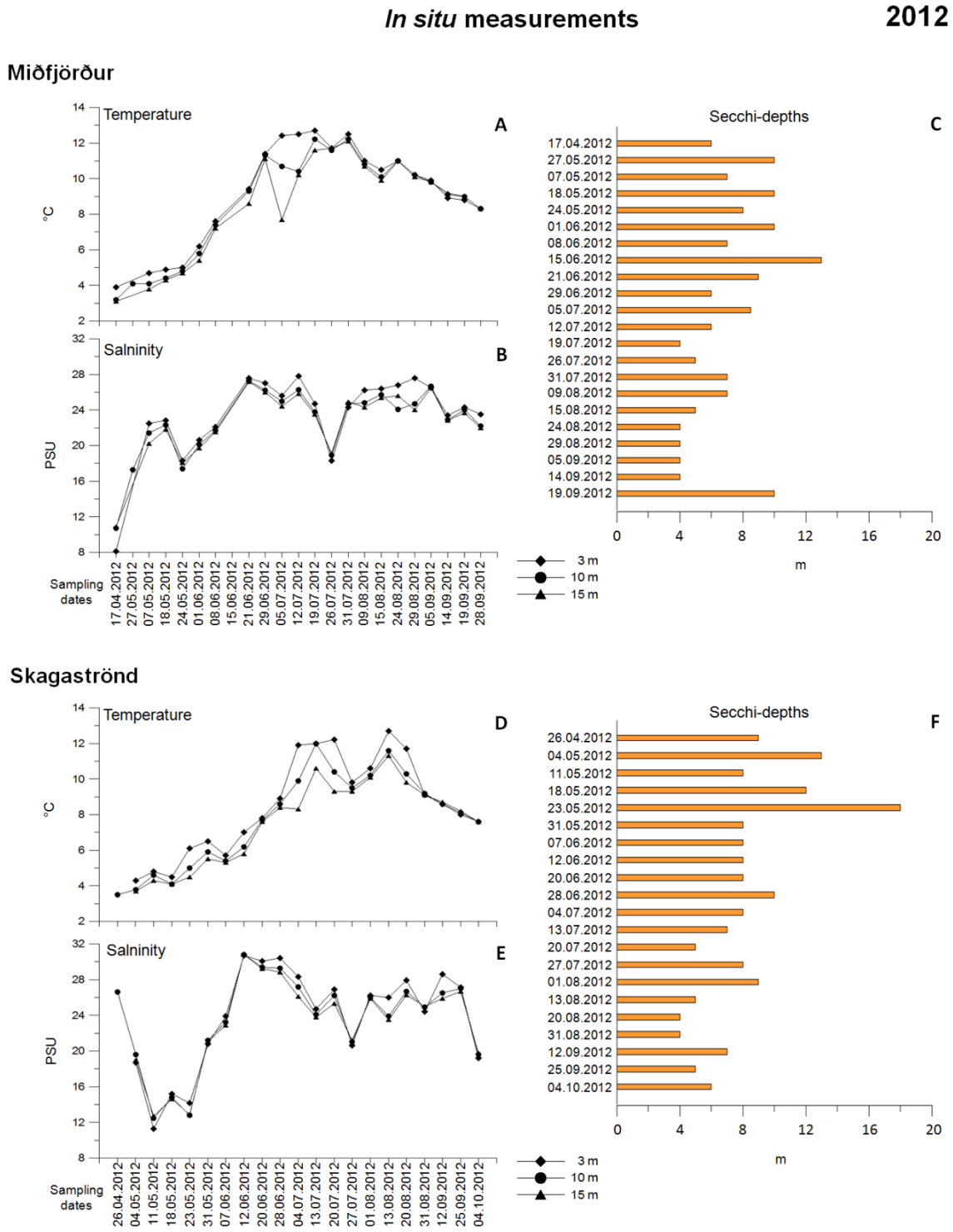


Figure17. Results of the *in situ* measurements (A, D: temperature, B, E: salinity, C, F: Secchi depths) at Miðfjörður and Skagaströnd in 2012.

Seasonal and spatial developments of the *in situ* parameters are given in Figures 16 and 17. The minimum temperatures, measured in 3 m depths at Miðfjörður and Skagaströnd, were found during spring 2011 (March/April: 1.2 and 4.2 °C, respectively), whereas the maximum temperature recorded was around 11.0 °C in July at both stations in the same depth and year (Figs. 16A and D). While spring temperatures did not vary significantly at both stations between 2011 and 2012, summer

temperatures in 2012 were up to 1.7 °C higher at both stations in comparison to those of 2012 ($ANOVA_{July2011/2012}$ $F_{1, 67}$ = 29.5, P < 0.001). Significant fluctuations in temperature depth profiles were also observed during summer 2011 and 2012. Particularly, a significant decrease of 18% between the 3 m and the 15 m temperature was recorded in Skagaströnd in July 2011 (ANOVA $F_{1, 68}$ = 30.1, P < 0.001). Furthermore, temperature differences at both stations occurred in 2012, being significant at Miðfjörður in July (38% difference between 3 m and 15m, ANOVA $F_{1, 68}$ = 22.8, P < 0.001, Fig. 17A).

Salinities varied in average between 22.8 and 25.6 PSU, sharply decreasing during and after rainfall and snow melting events and being highest during periods of dryness or only little rainfall (Figs. 16 and 17, B and E, respectively). The lowest salinity was observed at Miðfjörður in April 2012 (8.1 PSU, Fig. 17B), whereas the highest value was found at Skagaströnd in August 2011 (32.1 PSU, Fig. 16E). In addition, a small but significant decrease was recorded at Miðfjörður in May 2012 (19.7% $ANOVA_{July2011/2012}$ $F_{1, 33}$ = 27.9, P < 0.001, Fig. 17B). Average values of the salinity depth profiles varied only in narrow ranges over seasonal and spatial scales (5.1-6.4% differences). With the exception of higher averaged salinities at Miðfjörður in 3 m depth in 2011, overall, the highest averaged values were recorded in 10 m depth at both stations during 2011 and 2012. The general decline of salinities in 15 m depth at both stations during the course of sampling events is noteworthy.

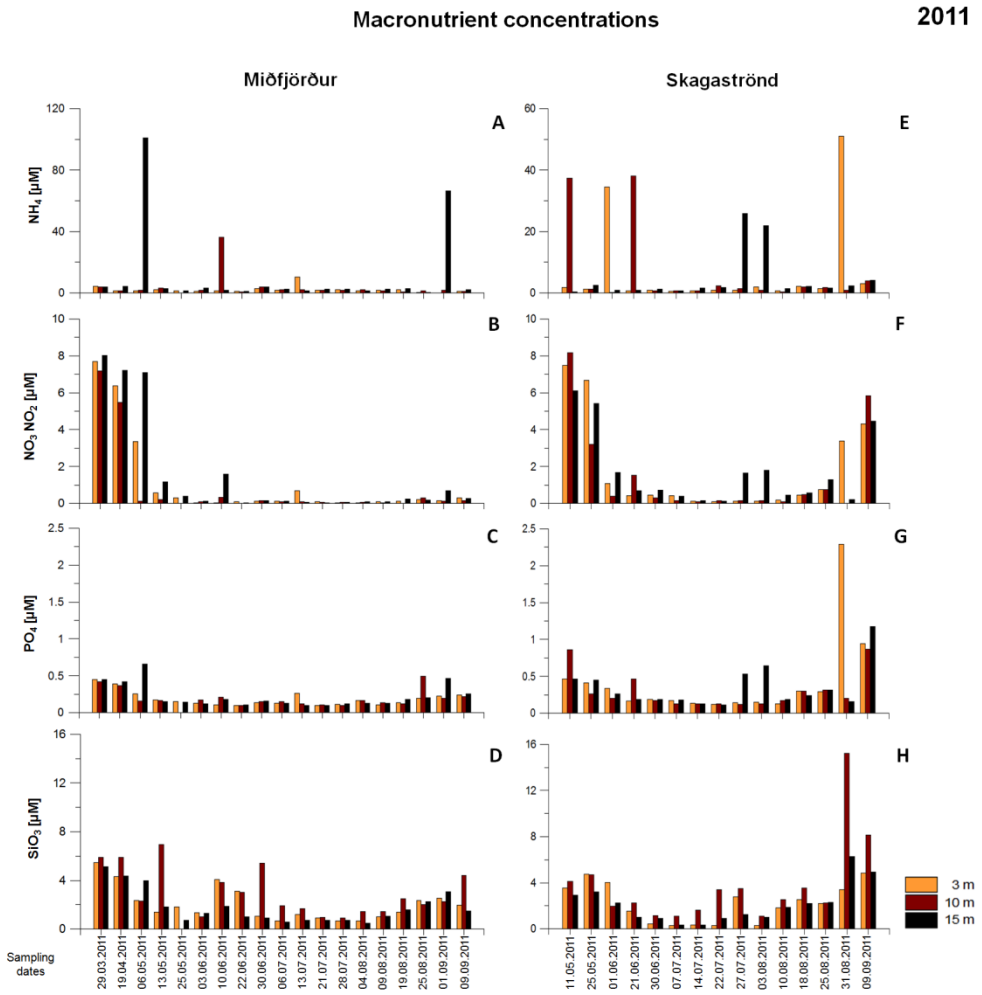


Figure18. Spatial and seasonal variations of macronutrient concentrations (A, E: ammonium, B, F: nitrate and nitrite, C, G: phosphate and D, H: metasilicate) at Miðfjörður and Skagaströnd in 2011 obtained from 3, 10 and 15 m depth.

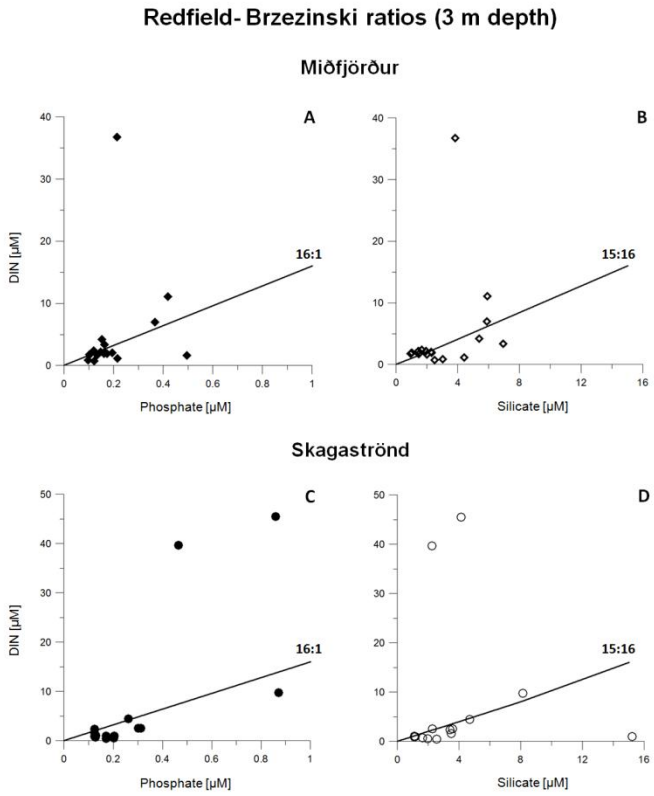


Figure19. Redfield-Brezezinski ratios of the 3 m depth N, P (A, C) and Si (B, D) relations at Miðfjörður and Skagaströnd in 2011. The ratio determines which nutrients are limiting in a localized system. According to Redfield (1934) the ratio for N:P is 16:1 (DIN = sum of ammonium, nitrate and nitrite). Diatoms need, among other nutrients, silicic acid to create biogenic silica for their frustules. As a result of this the Redfield-Brzezinski nutrient ratio was proposed for diatoms and stated to be C:Si:N:P = 106:15:16:1 (Brzezinski, 1985).

Seasonal and spatial trends in the clarity of the water column measured in terms of Secchi depths were also observed during the monitoring, showing only minor differences between the two stations in 2011 (6.7-7.3 m Secchi depths in average at Miðfjörður and Skagaströnd, respectively, Figs. 16C and F) and being significant in 2012 ($ANOVA_{2012ST/MID}$, $F_{1, 34} = 32.3$, $P < 0.001$, Figs. 17C and F). Generally, the depth of visibility was never less than 4 m, but could reach more than 10 m during the sampling events, being the highest at Skagaströnd in May 2012 (18 m, Fig. 17F).

Similar averages and high temporal variability in macronutrient concentrations were found over the course of sampling events in 2011 (Fig. 18). Overall, small but significant differences in the nutrient concentrations were present between the two stations ($ANOVA_{ammonium}$: $F_{1, 34} = 25.7$; $_{P}$: $F_{1, 33} = 26.5$; $_{Si}$: $F_{1, 34} = 25.1$, $P < 0.001$). In detail, ammonium concentrations varied in average from 0.7 µM in August to 3.8 µM in March at Miðfjörður (Fig. 18A) and ranged between 0.1 µM in June to 3.9 µM in September at Skagaströnd (Fig. 18E). The extraordinary high ammonium concentrations in May and September at Miðfjörður (up to 100.8 µM in 15 m depth) as well as in May, June, July and August at Skagaströnd in different depths (up to 50.9 µM in 10 m depth) are noteworthy, but were not considered in the statistical average analysis (c.f. Appendix). Depth profiles of averaged ammonium concentrations showed maximum values in 15 m depth at Skagaströnd (4.68 µM). The combined nitrate/nitrite concentrations exhibited seasonal trends, ranging from 20.01-0.1 µM in June to 7.2-8.1 µM in March/April at Miðfjörður and Skagaströnd, respectively (Figs. 18B, F). Averaged depths

profiles of nitrate and nitrite concentrations showed maximum values in 15 m depths at both stations (up to 55% higher than compared to 3 m and 10 m depth). Phosphate decreased steadily during spring and early summer (0.09 µM in June) and increased by the end of autumn (0.49-0.87 µM at Miðfjörður and Skagaströnd, respectively, Figs. 18C, G). In addition, averaged phosphate concentrations showed maximum values in 10 m depth at Skagaströnd (0.42 µM). Silicate concentrations ranged in average between 0.93 µM in late July to 5.91 µM in March at Miðfjörður and between 1.1 µM in mid July to 4.7 µM in May at Skagaströnd (Figs. 18D, H), whereas the averaged mean peaked in 3 m depth at both stations (2.9 µM, 10 and 15 m depth up to 30% lower). The extraordinary high silicate concentration in late August (15.2 µM, Fig. 18H) is also notable, but also not considered in the average analysis.

The N (here DIN = sum of ammonium, nitrate and phosphate) : P molar ratios in 3 m depth averaged by 10.3 and ranged from 0.6 to 86.0, being lowest in spring at Miðfjörður and highest in autumn 2008 at Skagaströnd (Fig. 19A, C). The DIN:DSi molar ratios ranged from 0.2 (Miðfjörður, Fig. 19B) to 1.8 (Skagaströnd, Fig. 19D). In addition, DIN:DSi molar ratios averaged 0.5, with significant differences between the seasons (ANOVA $F_{3,34} = 7.8$, $P < 0.001$).

Results Compendium

- **The depth of visibility** was never less than 4 m, but could reach more than 10 m during the sampling events, being the highest at Skagaströnd in May 2012.
- **Temperature** ranged in usual seasonal scales during the monitoring with significant temperature differences between 2011 and 2012 at both stations, whereas the **salinity** was generally much lower than expected for marine environments at least for the station at Skagaströnd.
- **The macronutrient data** obtained for 2011 indicated, besides obvious seasonal trends in nitrate and nitrite concentrations, only slightly seasonal differences. In addition, significant variations in the depth distributions were recorded.

4.2 Algal Biomasses (chl *a*), Total Abundances and Relative Composition of Potential Toxin Producing Taxa

The chl *a* concentrations, which represented all of the planktonic microalgal biomass varied considerably over the course of sampling events at both stations in 2011 and 2012 (Figs. 20A, D, G, J). While averaged chl *a* concentrations in 3 m depth ranged from 1.09-1.27 and 1.43-1.6 µg chl *a* L^{-1} at Skagaströnd and Miðfjörður in 2011 and 2012, respectively, maximum values were observed in May and June 2011 (5.13 and 1.74 µg chl *a* L^{-1}, Figs. 20A, D) as well as September and August 2012 (4.84 and 3.1 µg chl *a* L^{-1}, Figs. 20G, H). So overall, the chl *a* levels were higher during summer 2012 (July and August) than during summer 2011 (ANOVA: $F_{2,63} = 36.2$, $P < 0.0001$). In addition, significant seasonal differences were also found for the chl *a* values at Miðfjörður and Skagaströnd in September 2011 and 2012 (3 m depth). Here the chl *a* data showed a significant decline of 22% (2011, ANOVA $F_{1,142} = 25.9$, $P < 0.001$) and 31% (2012, ANOVA $F_{1,143} = 30.1$, $P < 0.001$) at Skagaströnd in comparison to the same sampling dates at Miðfjörður (± 3 days). Moreover, the comparison of the spatial depth distribution patterns showed seasonal differences between summer 2011 and spring

2012 at both stations ($ANOVA_{10\ m/15\ m;\ ST/MID}$: $F_{1,143}$ = 24.7, P < 0.0001), being distinctive among Skagaströnd and Miðfjörður in July and March (21.2 to 25.8%, respectively; ANOVA: $F_{1,143}$ = 30.1, P < 0.0001). Finally, the increase of chl *a* concentrations with depth at both stations in 2012 is noteworthy (up to 55% in 15 m depth at Skagaströnd; $ANOVA_{3m/10\ m/15\ m;\ ST/MID}$: $F_{1,132}$ = 24.7, P < 0.0001, Figs. 20G, J).

Total abundances of *Alexandrium* spp., *Dinophysis* spp. and *Pseudo-nitzschia* spp. showed significant differences in consideration of depth distributions as well as total cell numbers between the years 2011 and 2012 (Figs. 20B, E, H, K). Particularly, the difference in total cell numbers between 2011 and 2012 is noteworthy. Here, 40.4% lower total abundances were recorded in 2012 than compared to the previous year (average: 3.1 and 7.3 cells L^{-1}, regarding all three depths at both stations in 2012 and 2011). While differences in total cell numbers between the stations at Miðfjörður and Skagaströnd in 2012 were not significant (p > 0.05; average 2.9 and 3.1 cells L^{-1}, respectively, Figs. 20H, K), total abundances were significantly higher at Skagaströnd in 2011 (20%, $ANOVA_{MD/ST}$: $F_{1,102}$ = 25.5, P < 0.0001, Figs. 20B, E). Depth distributions of total abundances in 3, 10 and 15 m depth at Miðfjörður in 2011 and 2012 showed only slight variations, which were not statistically significant (p > 0.05). In contrast, the 3 and 10 m depth distribution varied significantly at Skagaströnd in both years. Especially, in 2011, total cell numbers were 30.7% higher in 10 m depth than compared to the 3 m data ($ANOVA_{ST\ 3m/10m}$: $F_{1,32}$ = 28.1, P < 0.0001, Fig. 20E).

Due to the restriction of the phototrophic zone and the location of the mussel cages in relation to the conducted toxin tests, all further analysis will focus on the 3 m depth distribution of abundances. Thus, in the figures 20C, F, I and L the relative abundances of the three genera of potential toxin producing taxa in relation to their seasonal occurrence are displayed for the monitoring in 2011 and 2012. Overall, the clear dominance of *Pseudo-nitzschia* spp. is most notable at both stations in 2011 and 2012, being the highest at Skagaströnd in 2011 with 87.8% of the total relative abundances (Fig. 20F). With the exception of slightly higher total relative abundances of *Alexandrium* spp. at Skagaströnd in 2011 (2.8%), *Dinophysis* spp. was the second most abundant group of the monitored taxa at both stations and years with total relative abundances ranging between 21.9% and 32.5% at Skagaströnd and Miðfjörður in 2012 (Figs. 20I, L). Consequently, *Alexandrium* spp. constituted the third group of the monitored taxa with total relative cell numbers varying from 7.5 to 15.5% at Skagaströnd and Miðfjörður in 2011 (Figs. 20C, F). Furthermore, the seasonal distribution of the genera showed also distinct differences between the stations and years of the monitoring. For example, *Dinophysis* spp. occurred in the highest relative abundances at Miðfjörður in August and September 2011 (Fig. 20C), whereas this genus had its highest occurrence between June and early August at the same station in 2012 (Fig. 20I).

Results compendium

- **The chl *a* concentrations** reflected only in minor scales expected seasonal trends and ranged overall from 1.09 to 5.13 µg L^{-1}. Extraordinary high chl *a* concentrations were recorded in 15 m depth.
- **Total and relative cell abundances** showed distinct seasonal and spatial differences in the occurrence of the three monitored taxa with significantly higher total abundances in 2011.
- **The clear dominance of *Pseudo-nitzschia* spp**. was most notable at both stations in 2011 and 2012, being the highest at Skagaströnd in 2011 with 87.8% of the total relative abundances.

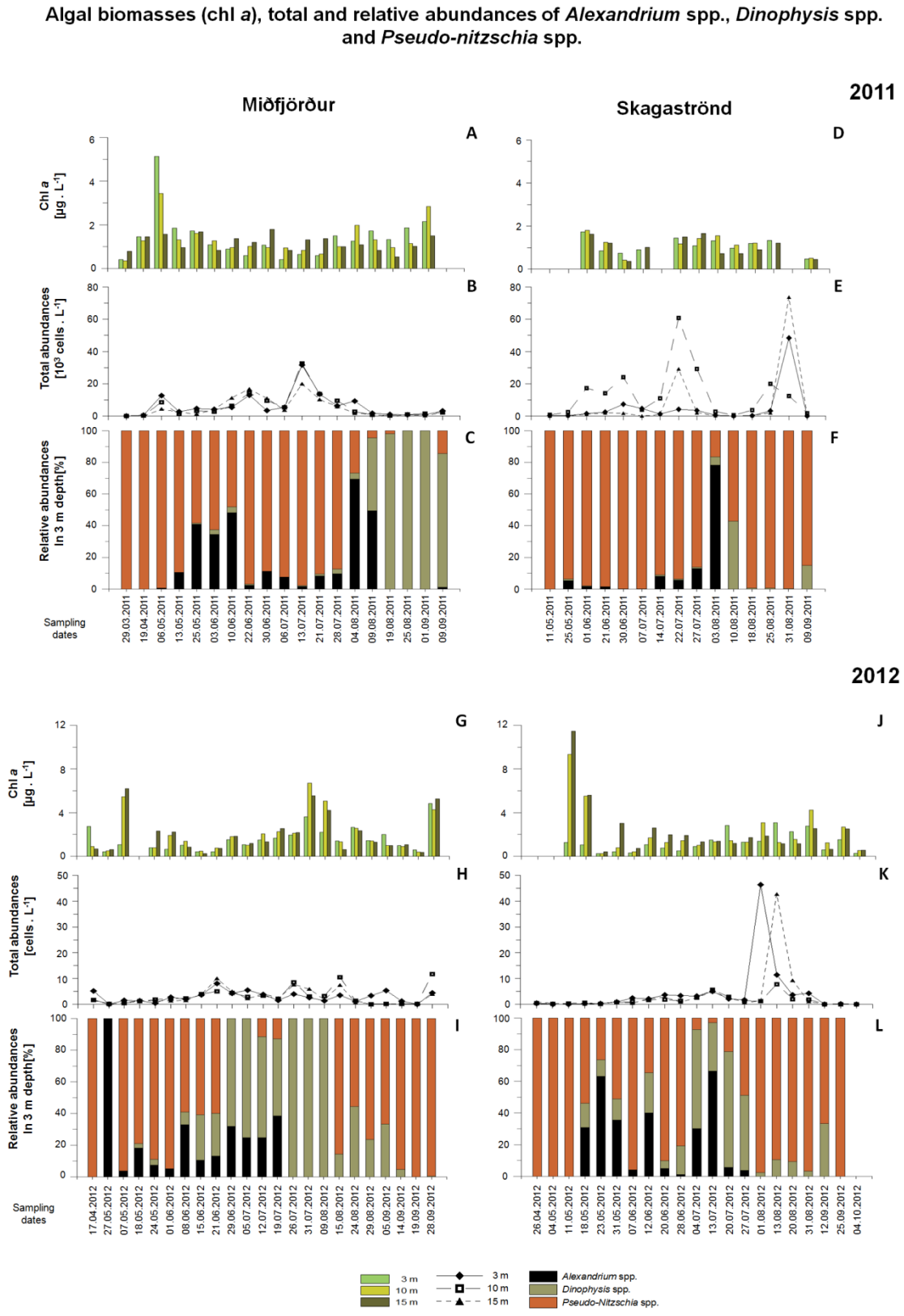


Figure 20. Spatial and seasonal variations of algal biomasses (measured as chl a; A and D), total abundances of *Alexandrium* spp., *Dinophysis* spp. and *Pseudo-nitzschia* spp. (given here for all three depths, B and E) and relative abundances of the three genera at 3 m depth (C and F) at Miðfjörður and Skagaströnd in 2011 and 2012.

4.3 Biotoxin Occurrences, Correlations and Composition

4.3.1 ASP Toxin Group

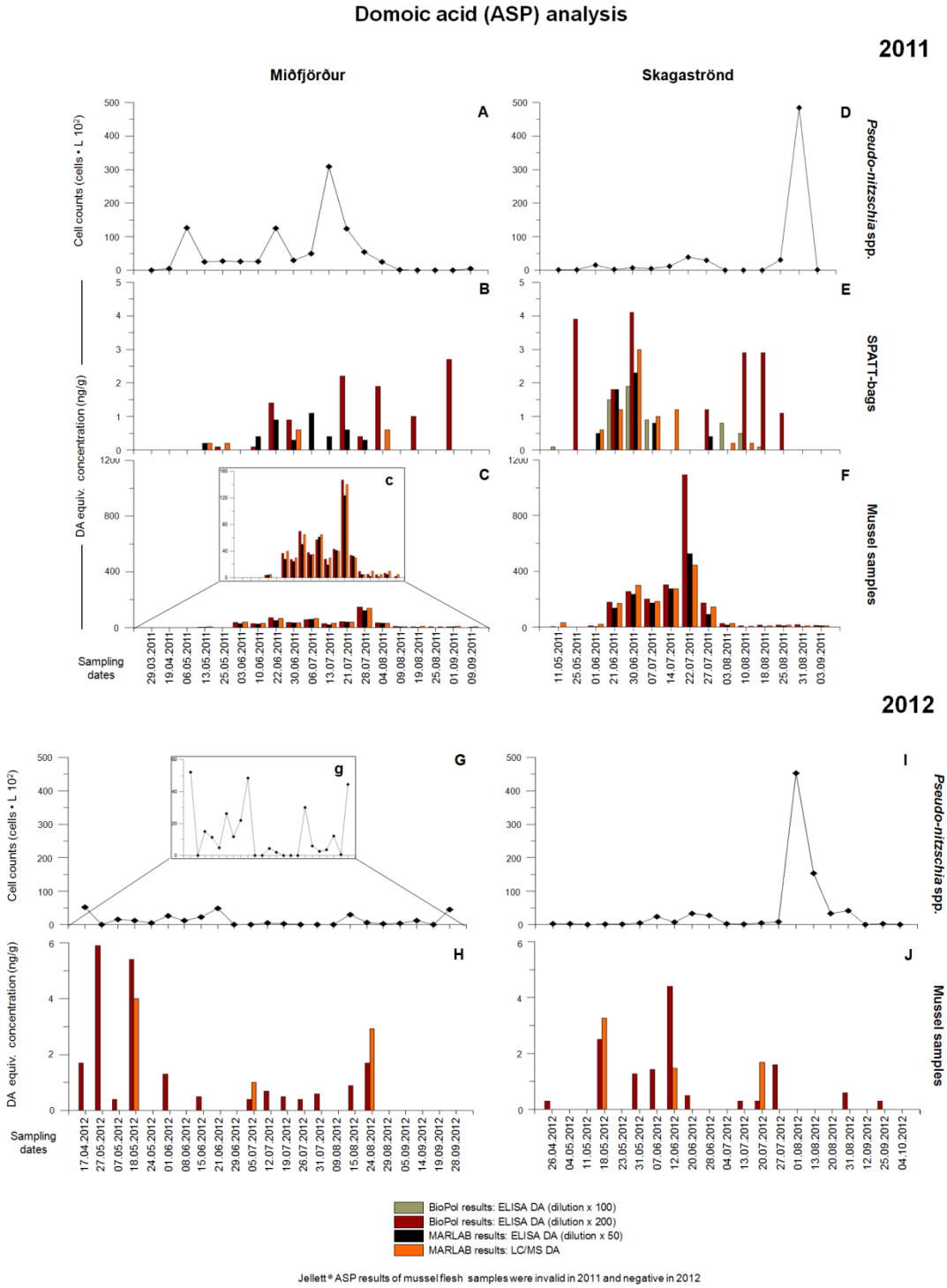


Figure 21. Seasonal and spatial variations of *Pseudo-nitzschia* spp. cell abundances (A, D, G, I) and domoic acid concentrations (DA equivalents, ASP toxins), tested in SPATT bags (2011 only, B, E) and mussel flesh (C, F, H, J) at Miðfjörður and Skagaströnd in 2011 and 2012. The small characters (c, g) indicate the same data, but in a larger scale. Tests were conducted once per sample without repetition, using Jellett® ASP (BioPol, ehf.), ASP Biosense® ELISA (BioPol, ehf. and MARLAB, Scotland, UK) and LC-MS/MS (MARLAB, Scotland, UK).

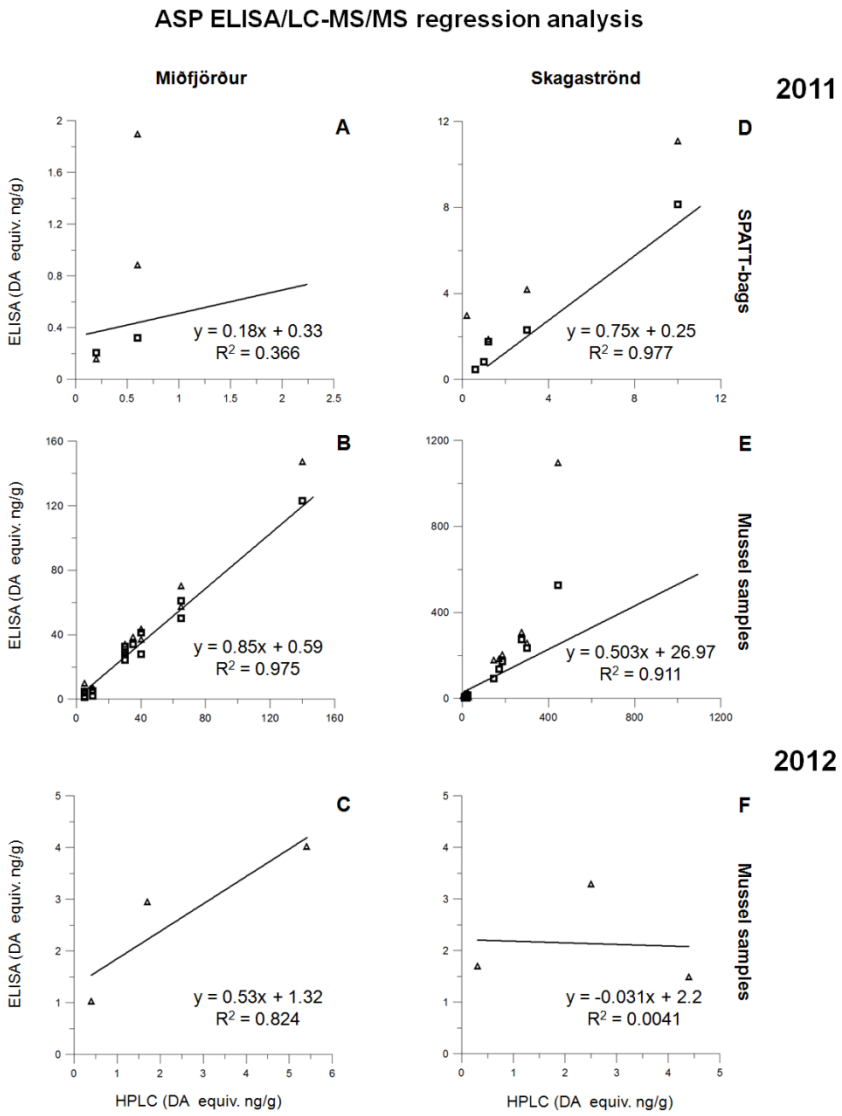


Figure 22. Comparison of total ASP toxin concentrations in SPATT bags (2011 only, A, D) and shellfish extracts (B, C, E, F) as determined by ASP Biosense® ELISA and LC-MS/MS obtained from the monitoring at Miðfjörður and Skagaströnd in 2011 and 2012.

Two different ASP tests were conducted with SPATT and shellfish samples during the monitoring in the years 2011 and 2012 (Fig. 21). Abundances of *Pseudo-nitzschia* spp. showed the highest values of 48.4 and 45.3 x 10^4 cells L^{-1} at Skagaströnd in August 2011 and 2012 (Figs. 21D, I), whereas in comparison with Skagaströnd (2011) the maximum cell densities at Miðfjörður were 36% and 98.9% lower in 2011 and 2012, respectively, (Fig. 21A, G). In the first year all Jellett® test results were invalid, the test results in the second year were negative. Furthermore, the ASP Biosense® ELISA test showed significant differences between the ASP concentrations in the SPATT bags and mussel samples, measured as domoic acid (DA) equivalents ($p > 0.05$). Here, DA concentrations were up to 98.2 % lower in the SPATT bags compared to the mussel samples, ranging between 0.1-2.7 (SPATT) and 3.8-146.7 (mussel flesh) ng g^{-1} at Miðfjörður (Figs. 21B, C) and 0.1-1.9 (SPATT) and 3.5-1093.2 (mussel flesh) ng g^{-1} at Skagaströnd (Figs. 21E, F). In contrast, DA concentrations in mussel samples from 2012 were on average 96% lower than compared to 2011 (Figs. 21H, J; the extraordinary high value at Skagaströnd in July 2011 was not considered in this calculation, since it was not confirmed

by the LC-MS/MS). Overall, 26 samples were recorded as failing the ELISA as the coefficient of variation (CV) from the analyses was determined to be above 20% (see Appendix). The presence of DA in all ELISA positive tested samples in 2011 was confirmed by LC-MS/MS (MARLAB) demonstrating an excellent correlation (r squared) of 0.81 in average ($p > 0.05$, Figs. 22A, B, D, and E). For 2012 only three mussel flesh samples were chosen according to the ELISA test results and analysed by LC-MS/MS. Here, the regression analysis showed for the samples from Miðfjörður an excellent correlation of 0.82 (r squared, Fig. 22C), whereas the correlation for Skagaströnd was only low (r squared = 0.0041, $p > 0.05$, Fig. 22F). Although generally lower cell numbers correspond with lower toxin concentrations ($p > 0.05$), the Pearson correlation of temporal toxin test results and abundances of *Pseudo-nitzschia* spp. were in all cases negative or not significant ($p < 0.05$, appendix).

Results compendium

- **Abundances of *Pseudo-nitzschia* spp.** showed the highest cell numbers at Skagaströnd in August 2011 and 2012 (48.4 and 45.3 x 10^4 cells L^{-1}) and were not significantly correlated to the toxin measurements.
- **Jellett® test results** were negative in 2012 (invalid 2011), whereas **the ASP Biosense® ELISA test** showed values ranging between 0.1-2.7 (SPATT) and 3.8-146.7 (mussel flesh) ng g^{-1} at Miðfjörður and 0.1-1.9 (SPATT) and 3.5-1093.2 (mussel flesh) ng g^{-1} at Skagaströnd in 2011 and 2012, respectively.
- **The presence of DA** in all ELISA positive tested samples in 2011 was confirmed by HPLC (MARLAB) demonstrating an excellent correlation (r squared) of 0.81 in average.

4.3.2 DSP Toxin Group

SPATT and shellfish samples were analysed for the presence of DSP toxins in the monitoring 2011 and 2012, using hydrolysed and non-hydrolysed samples in the Jellett® and PP2A OkaTest® as well as for data comparison and toxin composition LC-MS/MS (Fig. 23). Abundances of *Dinophysis* spp. showed the highest occurrence at Miðfjörður in June 2012 (4.08 10^3 cells L^{-1}, Fig. 23G), whereas cell numbers in 2011 were 33.3% lower (September 2.72 10^3 cells L^{-1}, Fig. 23A). In comparison with the maximum abundances at Miðfjörður in June 2012, cell numbers were about 50% lower at Skagaströnd during the same months and year (Fig. 23I) and up to 75% lower in September 2011 (Fig. 23D). While all Jellett® DSP tests gave errors in the analysis of mussel extracts in 2011, the tests showed positive results in 15 out of 23 hydrolysed shellfish samples from Miðfjörður and 11 out of 21 from Skagaströnd in 2012 (results are added to the Figs. 23G, I).

The analysis of the total DSP toxin concentrations in mussel samples, using the PP2A enzyme based OkaTest®, showed generally significantly lower concentrations for 2011 compared to the 2012 samples ($p > 0.05$). In detail, the highest values obtained from the analysis of the hydrolysed shellfish extracts were 139.4-293.0 ng g^{-1} at Skagaströnd and Miðfjörður in 2011 (Figs. 23C, F), with four (13) samples which were under the limit of detection (<63 ng/g) at Skagaströnd and six (12) ones at Miðfjörður (the numbers in brackets indicate the MARLAB results).

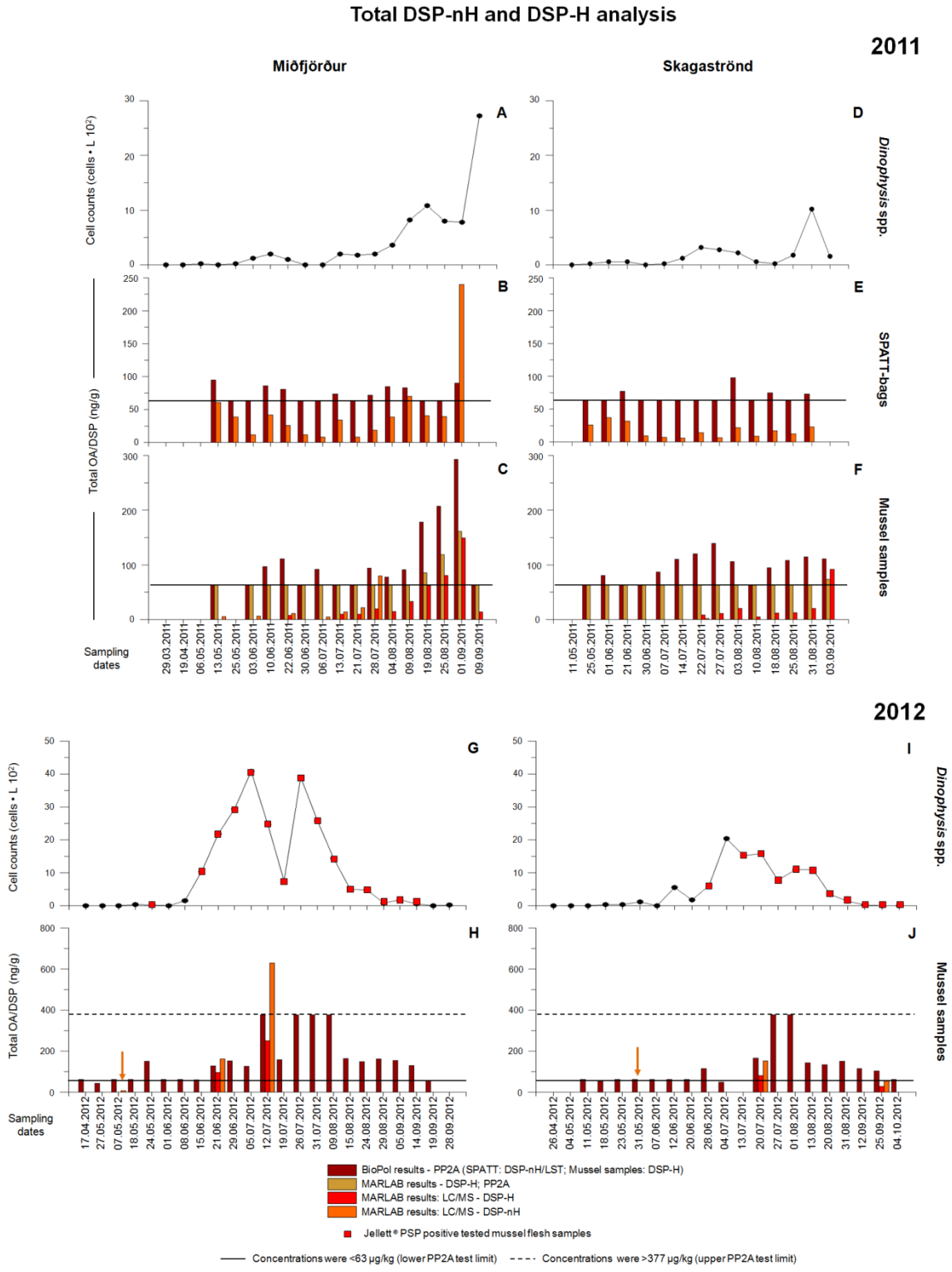


Figure 23. Seasonal and spatial variations of *Dinophysis* spp. cell abundances (A, D, G, I) and total DSP toxin (PP2A) concentrations, analysed in the water column in 2011 using SPATT-bags (B, E) and mussel flesh (C, F, H, J) at Miðfjörður and Skagaströnd in 2011 and 2012. Tests were conducted once per sample without repetition, using Jellett® DSP (hydrolysed samples, BioPol, ehf.), the PP2A OkaTest® (BioPol, ehf. and MARLAB, Scotland, UK) and LC-MS (MARLAB, Scotland, UK). The OkaTest® and LC-MS analysis were performed with hydrolysed and non-hydrolysed samples.

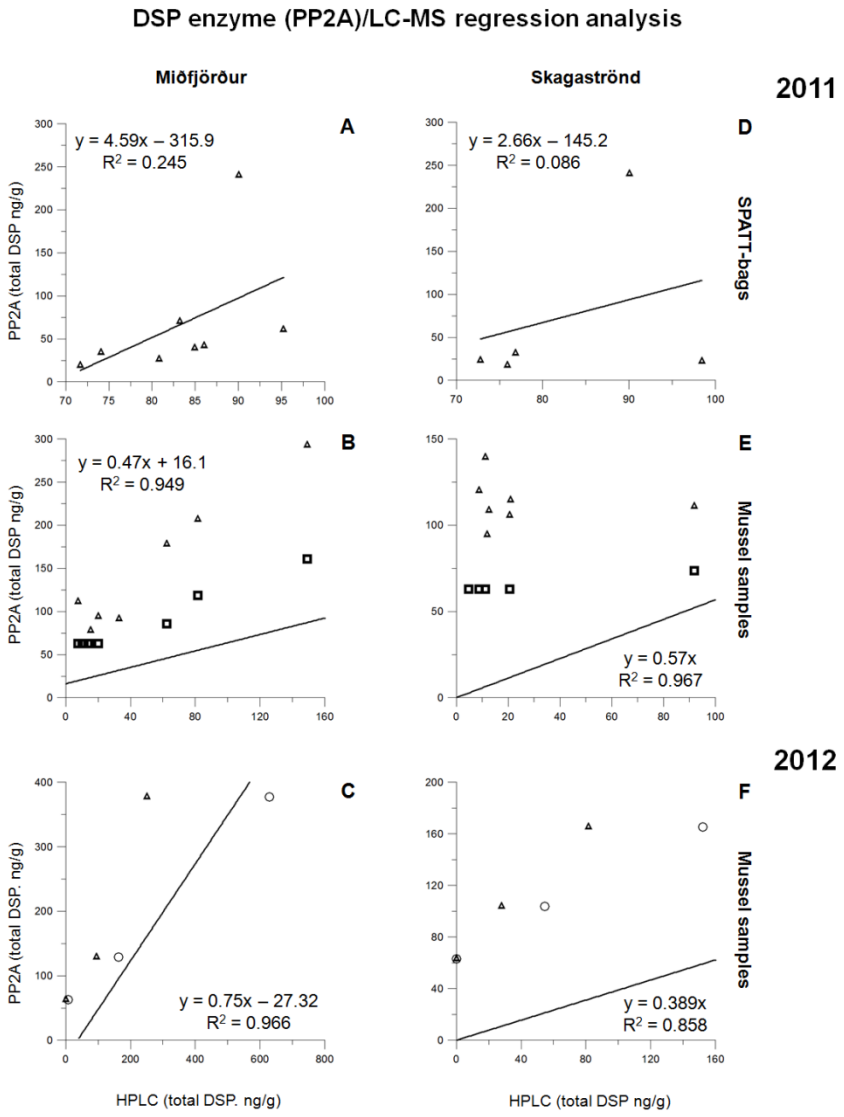


Figure 24. Comparison of total DSP toxin concentrations in SPATT bags (2011 only, A, D) and shellfish extracts (B, C, E, F) as determined by PP2A OkaTest® and LC-MS obtained from the monitoring at Miðfjörður and Skagaströnd in 2011 and 2012.

In contrast, total DSP concentrations in hydrolysed shellfish extracts were four times over the limit of detection (>377 ng/g) out of overall 22 analysed samples from Miðfjörður and two times out of 18 samples from Skagaströnd in 2012 (Figs. 23H, J). The LC-MS results confirmed this observation and showed for the value on the 10th of July 2012 a peak total DSP concentration of 629.6 ng g^{-1} (Fig. 23H).

In addition, the highest values obtained from the analysis of the non-hydrolysed SPATT bags samples were 95 and 98 ng g^{-1} at Skagaströnd and Miðfjörður, respectively, with seven samples under the limit of detection at Skagaströnd and nine ones at Miðfjörður (Figs. 23B, E). The comparison of PP2A assays and LC-MS results showed excellent correlations in the analysis of the mussel samples in 2011 and 2012 (r squared = 0.935 in average, Figs. 24B, C, E, F), whereas the results of the SPATT samples were only weakly correlated to the LC-MS results (Figs. 24A, D). Similarly as observed for the relationship of total abundances and toxin concentrations in the ASP monitoring, low total DSP concentrations were also positive correlated with lower abundances of the

potential toxin producing species ($p > 0.05$). In contrast, the temporal analysis of the single dates *versus* the abundance of *Dinophysis* spp. in relation to toxin concentrations showed no positive or significant correlations ($p < 0.05$; data in appendix).

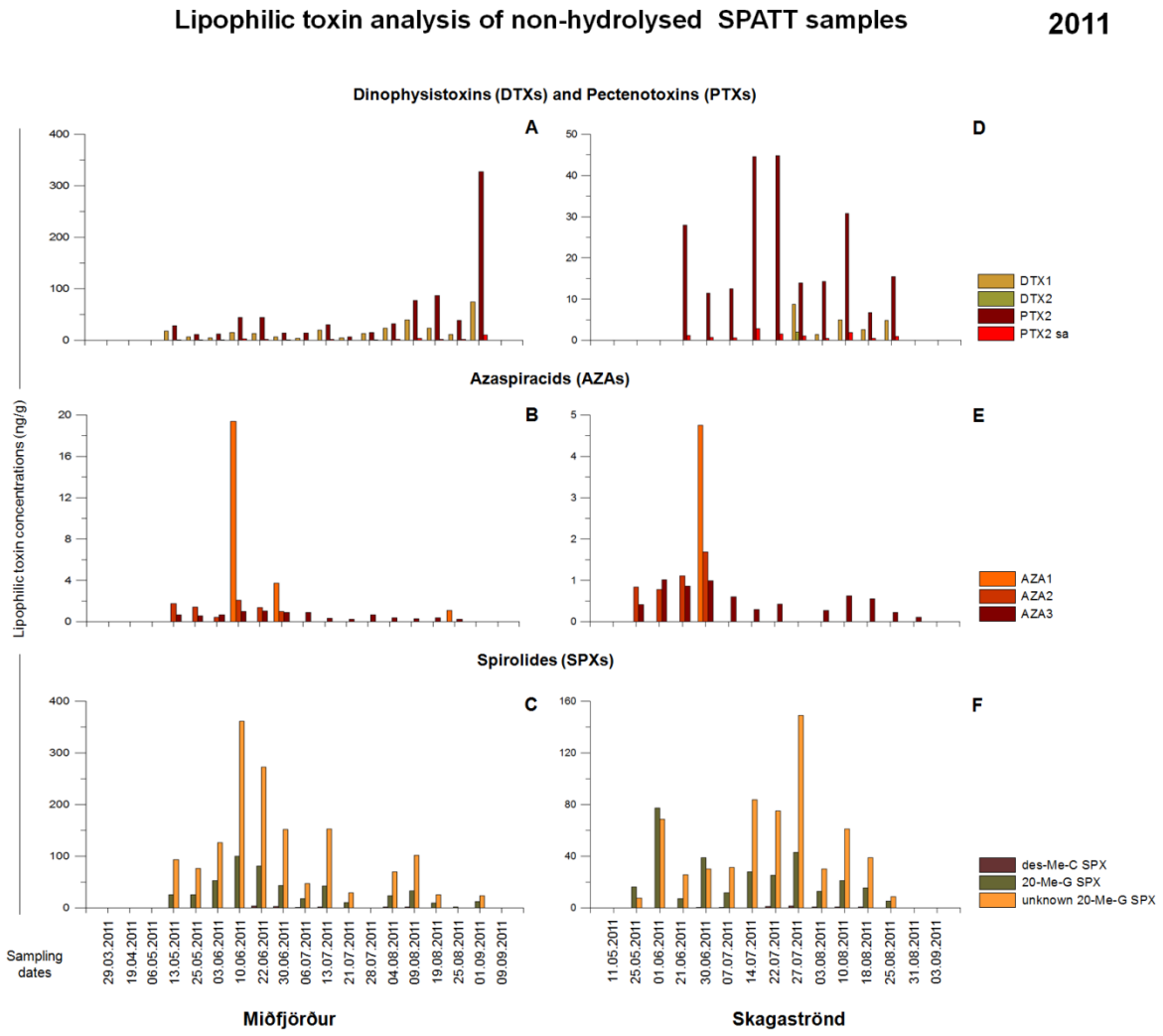


Figure 25. Seasonal and spatial variations of lipophilic toxin concentrations in the water column (SPATT-bags, non-hydrolysed samples) analysed by LC-MS at Miðfjörður and Skagaströnd in 2011. Differentiated were: A) Dinophysistoxins (DTXs) and Pectentotoxins, B) Azaspiracids (AZAs) and C) Sprolides (SPXs). Analyses were conducted by MARLAB (Scotland, UK).

The composition of the DSP toxins, obtained from the LC-MS/MS analysis of non-hydrolysed SPATT and mussel samples, showed all the toxins of the lipophilic family were observed during the period from 2011 to 2012, except for the yessotoxins such as 45-OH YTX and homo YTX (Figs. 25 and 26). Apart from okadaic acid (OA) and dinophysistoxins (DTX), which were present throughout the monitoring, pectentotoxins (PTX), spirolides (SPX) and azaspiracids (AZA) were also recorded with distinct differences in the accumulation in SPATT bags and in the shellfish samples. Significant relations were found for the pectentotoxins PTX2 and it's in the shell fish metabolized form PTX2 seco acid (sa) in the samples from Miðfjörður and Skagaströnd, showing strong positive correlations ($r^2 = 0.703$ and 0.645 at Miðfjörður and Skagaströnd in 2011, respectively ($p > 0.05$, Table appendix, Figs. 25 and 26A, D). In 2012, only the presence of PTX2 sa in three out of six samples shellfish flesh could be confirmed by the LC-MS analysis, whereas PTX1 and 2 were not detected (Table 4).

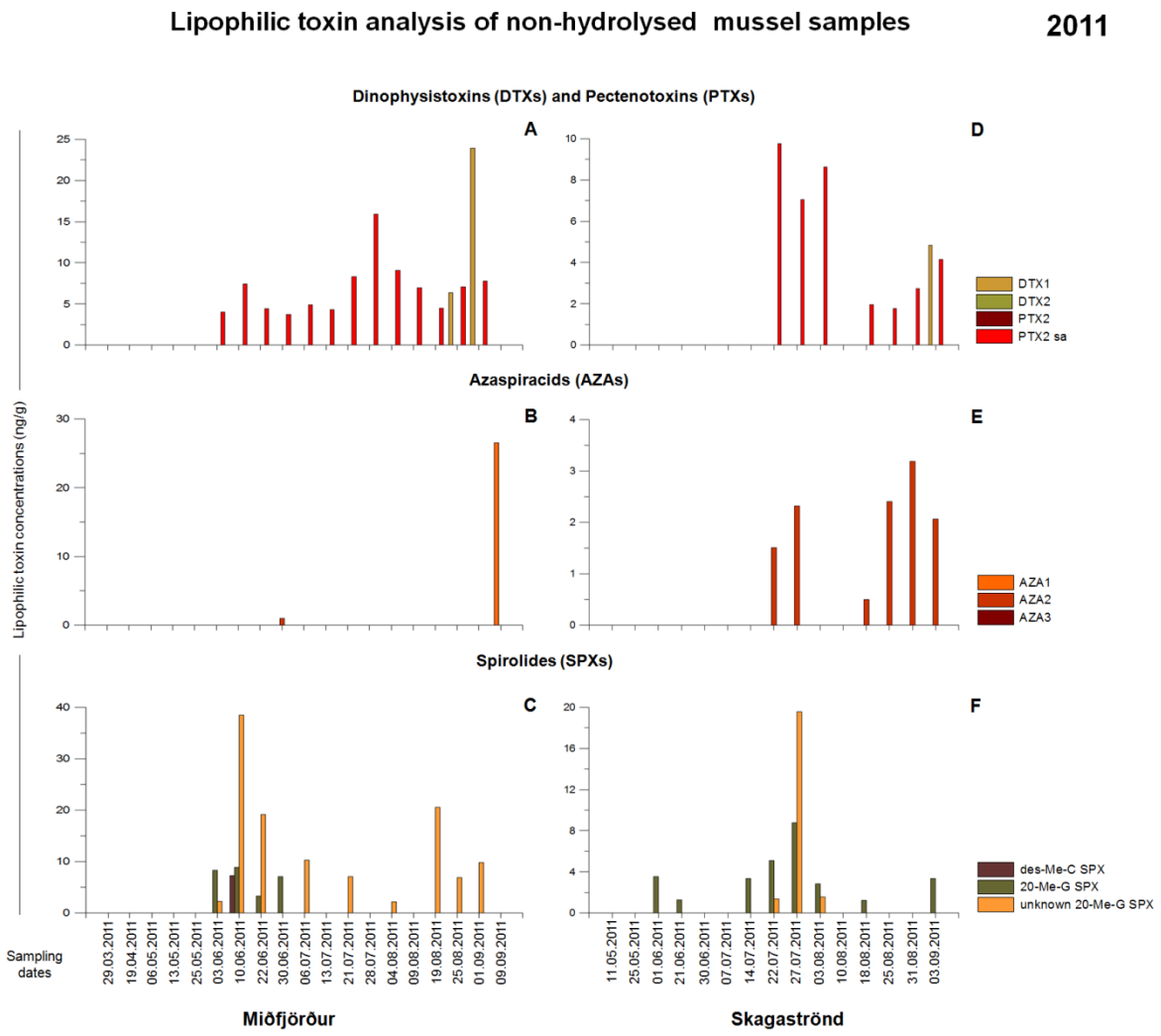


Figure 26. Seasonal and spatial variations of lipophilic toxin concentrations analysed by LC-MS in non-hydrolysed mussel samples obtained from Miðfjörður and Skagaströnd in 2011. Differentiated were: A) Dinophysistoxins (DTXs) and Petentotoxins, B) Azaspiracids (AZAs) and C) Sprolides (SPXs). Analyses were conducted by MARLAB (Scotland, UK).

Table 3. Toxin profiles 2012 part I: Analysis of the Dinophysistoxins (DTX) obtained from hydrolysed and non-hydrolysed mussel samples. Analyses were conducted by MARLAB (Scotland, UK).

Toxins / Sampling date	non-hydrolysed		hydrolysed	
	DTX-1 [µg/kg]	DTX-2 [µg/kg]	DTX-1 [µg/kg]	DTX-2 [µg/kg]
Miðfjörður				
07.05.12	<LOD	<LOD	20.9	<LOD
21.06.12	47.1	<LOD	42.3	<LOD
12.07.12	188.2	<LOD	222.2	<LOD
Skagaströnd				
31.05.12	<LOD	<LOD	<LOD	<LOD
20.07.12	102.9	<LOD	96.1	<LOD
25.09.12	33.9	<LOD	64.5	<LOD

Table 4. Toxin profiles 2012 part II: Analysis of non-hydrolysed mussel samples for the presence of lipophilic toxins. Analyses were conducted by MARLAB (Scotland, UK).

Toxins / Sampling date	Azaspiracids (AZAs)				
	AZA-1 [µg/kg]	AZA-2 [µg/kg]	AZA-3 [µg/kg]	AZA-4 [µg/kg]	AZA-5 [µg/kg]
Miðfjörður					
07.05.12	<LOD	<LOD	<LOD	<LOD	0.1
21.06.12	<LOD	<LOD	<LOD	0.1	0.2
12.07.12	0.7	<LOD	<LOD	<LOD	0.2
Skagaströnd					
31.05.12	0.1	0.1	0.1	0.2	0.2
20.07.12	0.4	0.4	<LOD	0.1	0.1
25.09.12	0.1	<LOD	<LOD	0.1	0.1

Toxins / Sampling date	Pectentotoxins (PTXs)* and Spirolides (SPXs)					
	PTX2	PTX1	PTX2 sa	epi PTX2 sa	des-Me-C SPX [µg/kg]	20-Me-G SPX [µg/kg]
Miðfjörður						
31.05.12	–	–	–	–	0.3	1.4
20.07.12	–	–	+	–	0.8	5.7
25.09.12	–	–	+	+	0.4	2.3
Skagaströnd						
07.05.12	–	–	–	–	0.2	4.6
21.06.12	–	–	+	+	0.5	4.3
12.07.12	–	–	–	–	0.4	3.2

*Only qualitative results for some toxins due to lack of standards (e.g. PTX2 sa & epi PTX2 sa) – Presence = [+], Absence = [–]

Several other significant correlations between SPATT and mussel samples could be identified in the statistical analysis, from which AZA2 in samples at Miðfjörður in September (Figs. 25, 26B) and AZA3 at Skagaströnd in August 2011 (Figs. 25, 26E) were most important (r^2 = 0.651 and 0.603 at Miðfjörður and Skagaströnd in 2011, respectively; $p > 0.05$, Table appendix). Furthermore, the high concentrations in the SPATT samples (up to 360.9 ng g^{-1} in August 2011, Fig. 25C) and the corresponding accumulation in shell fish samples of the spirolides 20-Me-G SPX as well as the unknown 20-Me-G SPX are most notable (Figs. 25, 26C, F).

Results compendium

- **Abundances of *Dinophysis* spp**. showed the highest occurrence at Miðfjörður in June 2012. Highest abundances were not or negative correlated to the DSP toxin concentrations, but seemed to precede the toxin events.
- While all **Jellett® DSP** tests were invalid in 2011, the tests showed several positive results in 2012. The comparison of the **OkaTest®and LC-MS/MS results** showed excellent correlations in the analysis of the mussel samples in 2011 and 2012.
- **The DSP toxin profiles** showed most of the toxins of the lipophilic family were present during the monitoring. In this context, the differences between SPATT and mussel flesh samples as well as the occurrence of **azaspiracids** and **an unknown spirolide** are noteworthy**.**

4.3.3 PSP Toxin Group

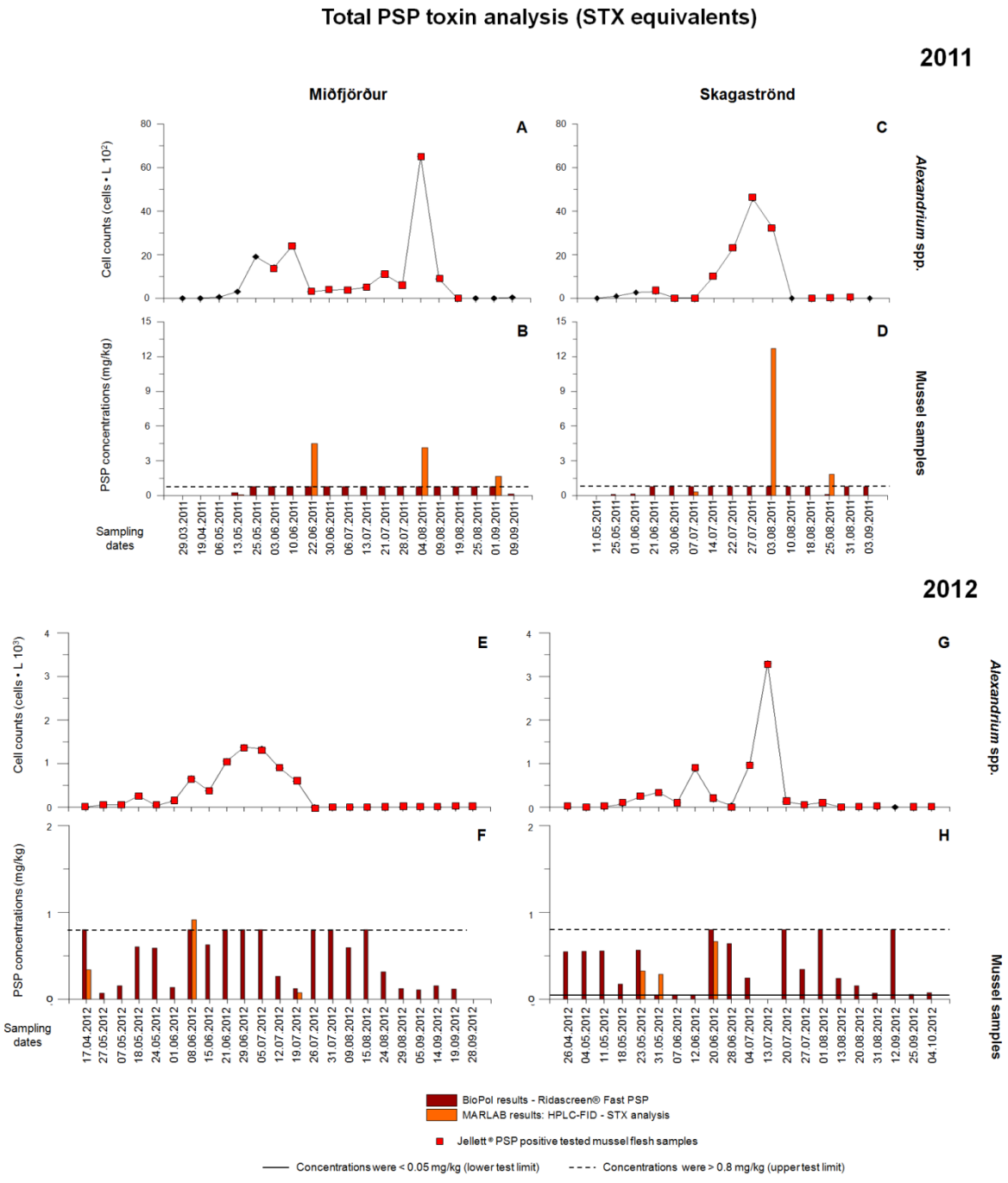


Figure 27. Seasonal and spatial variations of *Alexandrium* spp. cell abundances (A, C, E, G) and PSP toxin (STX equivalents) concentrations, analysed in mussel flesh) at Miðfjörður and Skagaströnd in 2011 ((B, D) and 2012 (F, H). Tests were conducted once per sample without repetition, using Jellett® PSP (BioPol, ehf.), the Ridascreen® Fast PSP CELISA (BioPol, ehf,) and HPLC (MARLAB, Scotland, UK).

Shellfish samples only were analysed for the presence of PSP toxins in the monitoring 2011 and 2012, using acidic samples in the Jellett® PSP, and Ridascreen® Fast PSP cELISA and also for data comparison and PSP toxin composition LC-MS/MS (Fig. 27). Abundances of *Alexandrium* spp. were the highest at Miðfjörður in 2011 (6.5 x 10^3 cells L^{-1}, Fig. 27A). All other maxima in the years 2011

(Skagaströnd: 84.3%, Fig. 27C) and 2012 (both stations: 78.0% at Miðfjörður and 68.6% at Skagaströnd, Figs. 27E, G) were significantly lower (p > 0.05). In the pre-screening Jellett® PSP test results gave 11 out of 19 samples from Miðfjörður and ten out of 15 samples from Skagaströnd positive results in 2011. In 2012, all samples from Miðfjörður and 20 out of 21 samples from Skagaströnd were positive when tested with the Jellett® PSP test (results are also displayed in Figs. 27A, C, E, G).

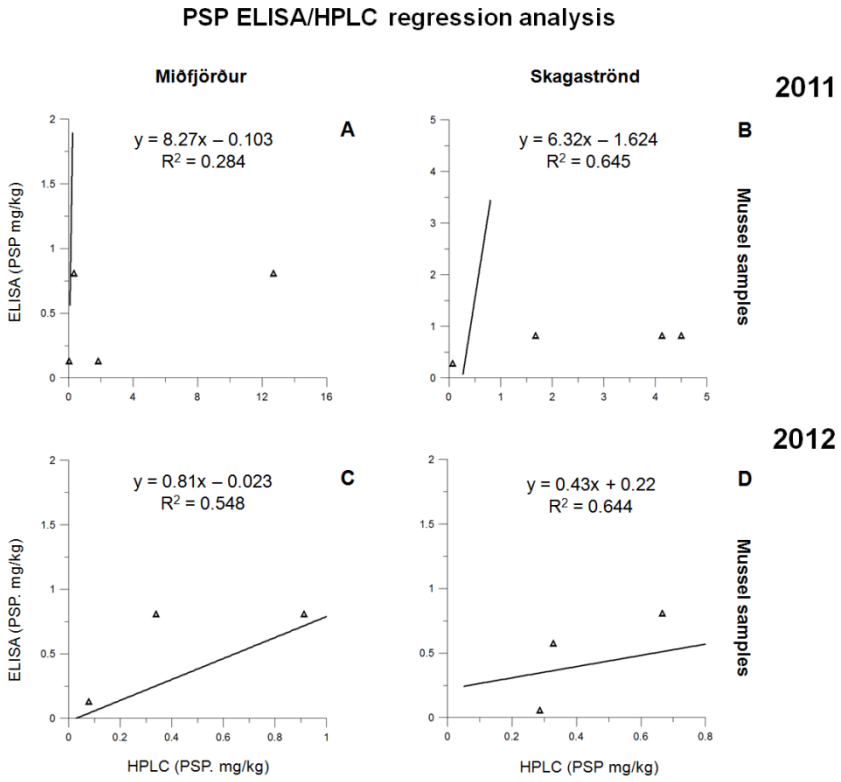


Figure 28. Comparison of total PSP toxin concentrations in shellfish extracts as determined by Ridascreen® Fast PSP cELISA and HPLC obtained from the monitoring at Miðfjörður and Skagaströnd in 2011 (A, B) and 2012 (C, D).

All tested shellfish samples showed in the cELISA test positive results during the monitoring in 2011 and 2012 at both stations (Fig. 27B, D, F, H). Thereby the PSP concentrations, measured in terms of Saxitoxin equivalents (STX), reached several times the upper test limit (> 0.8 mg kg^{-1}). Overall, significantly lower PSP concentrations were observed in shell fish samples from 2012 compared to the samples from 2011 (p > 0.05), ranging between 0.053 mg kg^{-1} mussel flesh (or lower) and the upper limit of detection (LOD). In detail, 73.7 and 73.3% of the mussel samples at Miðfjörður and Skagaströnd, respectively, were over the limit of detection in 2011 (>LOD, Figs. 27B, D), whereas only 34.8 and 19.1% of the samples reached this limit in 2012 (Figs. 27F, H). In addition, four samples from Skagaströnd lay under the limit of detection in 2012 (<LOD, 0.05 mg kg^{-1}, Fig. 27H). A total of eight samples in 2011 (four from each station) and six samples from 2012 (three samples from each station) were further analysed by HPLC. Here the highest PSP concentration was recorded at Skagaströnd in August 2011 (12.7 mg STX diHCL eq. kg^{-1}, Fig. 27D), corresponding to the >LOD measured in the cELISA test. The composition of the PSP toxins in 2011 and 2012 showed the presence of the gonyautoxins GTX2 and GTX3 in all analysed shellfish samples, constituting beside

STX the major part of the extraordinary high PSP concentration in August 2011 mentioned above (Tables 5 and 6, Fig. 27D). In contrast, other compounds such as GTX1 (75% of the samples in 2011, 0% in 2012), GTX4 (62.5% in 2011, 50% in 2012) and also STX (87.5% in 2011, 100% in 2012) were more heterogeneous distributed and not found in all samples. In this context, the occurrence of the gonyautoxin derivate dc-GTX-3 as well as of the saxitoxin derivatives N-1-hydroxysaxitoxin (NEO) and dc-STX in some of the mussel samples in 2011 is noteworthy (Table 5). Although in most cases a rough accordance between the >LOD and HPLC results in comparison among the cELISA tests and HPLC analysis was observable, the regression analysis gave only weak correlations between both, ranging from 0.416 to 0.644 (r squared in average) at Miðfjörður and Skagaströnd, respectively (Fig. 28), which were not statistically significant (p<0.05). Finally, the statistical analysis of the relationship between the cell counts of *Alexandrium* spp. and the levels of PSP concentrations showed positive significant correlations for only two dates in 2011 and 2012 at Miðfjörður (04.08.2011: r^2 = 0.523 and 29.06.2012: r^2 = 0.488, p > 0.05). All other correlations between both were either negative or not significant (p < 0.05, Appendix).

Table 5. PSP toxin profiles 2011: Analysis of mussel samples for the presence of GTX and STX toxins. Analyses were conducted by MARLAB (Scotland, UK).

Toxins / date	Gonyautoxins (GTXs) and Saxitoxins (STXs)								
	GTX1 [mg/kg]	GTX2 [mg/kg]	GTX3 [mg/kg]	GTX4 [mg/kg]	dc-GTX-2 [mg/kg]	dc-GTX-3 [mg/kg]	NEO [mg/kg]	dc-STX [mg/kg]	STX [mg/kg]
Miðfjörður									
13.05.11	–	0.025	0.026	–	–	–	–	–	0.018
03.06.11	0.077	1.493	0.812	0.014	–	0.003	0.035	–	2.063
06.07.11	0.295	0.840	1.074	0.095	–	–	0.081	0.009	1.729
28.07.11	0.005	0.628	0.327	0.030	–	–	–	–	0.690
Skagaströnd									
25.05.11	–	0.009	0.006	–	–	–	–	–	–
07.07.11	0.014	0.082	0.102	–	–	–	–	–	0.118
03.08.11	0.600	3.431	2.717	0.123	–	–	0.168	–	5.660
25.08.11	0.151	0.645	0.370	0.028	–	–	–	–	0.650

Table 6. PSP toxin profiles 2012: Analysis of mussel samples for the presence of GTX and STX toxins. Analyses were conducted by MARLAB (Scotland, UK).

Toxins / date	Gonyautoxins (GTXs) and Saxitoxins (STXs)								
	GTX1 [mg/kg]	GTX2 [mg/kg]	GTX3 [mg/kg]	GTX4 [mg/kg]	dc-GTX-2 [mg/kg]	dc-GTX-3 [mg/kg]	NEO [mg/kg]	dc-STX [mg/kg]	STX [mg/kg]
Miðfjörður									
17.04.12	–	0.104	0.136	–	–	–	–	–	0.099
08.06.12	–	0.199	0.397	–	–	–	–	–	0.313
19.07.12	–	0.023	0.028	–	–	–	–	–	0.024
Skagaströnd									
23.05.12	–	0.080	0.119	0.014	–	–	–	–	0.113
31.05.12	–	0.021	0.125	0.123	–	–	–	–	0.016
20.06.12	–	0.145	0.261	0.028	–	–	–	–	0.231

Abbreviations: NEO: N-1-hydroxysaxitoxin.

Results compendium

- **Abundances of *Alexandrium* spp.** were the highest at Miðfjörður in 2011. The Pearson analysis of abundances and toxin concentrations showed positive significant correlations for two dates.
- **Jellett® PSP test results** gave positive results in 57.9 and 66.7% as well as 100% and 95.2% of the samples from Miðfjörður and Skagaströnd in 2011 and 2012, respectively.
- All tested shellfish samples showed in the **Ridascreen® Fast PSP cELISA** test positive results during the monitoring, reaching several times the upper test limit. Although in most cases a rough accordance between the >LOD and LC-MS results in **comparison among the cELISA tests and LC-MS analysis** was observable, the regression analysis gave only weak correlations between both.
- **The composition of the PSP toxins** in 2011 and 2012 showed the presence of the gonyautoxins GTX2 and GTX3 in all analysed shellfish samples, constituting beside STX the major part of the extraordinary high PSP concentration in August 2011. In this context, the occurrence of the gonyautoxin derivate dc-GTX-3 as well as of the saxitoxin derivatives N-1-hydroxysaxitoxin (NEO) and dc-STX in some of the mussel samples in 2011 is noteworthy.

4.4. Relationship between Environmental Data, Abundances and Biotoxin Occurrences – Canonical Correspondence Analysis (CCA)

Several environmental variables were closely correlated with the genus group matrix in relation to the toxin groups according to canonical correspondence analysis (CCA) of potential toxin producing taxa occurrence at Miðfjörður and Skagaströnd in 2011 and 2012 (Fig. 29). The first two CCA axes explained 85.5% and 80.3% of the variance at Miðfjörður in 2011 and 2012 (Fig. 29A, B) as well as 80.5% and 82.6% at Skagaströnd 2011 and 2012, respectively (Fig. 29C, D). In all four sceneries, the first CCA axis was positively correlated with temperature and day length and negatively associated to salinity. Temperature and day length accounted for up to 19% of the overall variance among the environmental variables. In addition, the first CCA axis was negatively associated with NH_4^+ and NO_3^-/ NO_2^- at Miðfjörður in 2011, whereas at Skagaströnd in 2011 only NH_4^+ showed a negative correlation. Due to the lack of nutrient data, a different distribution of negative associated variables is found for both stations in 2012. Here with the second CCA axis the variables salinity and Secchi depth at Miðfjörður and Skagaströnd, respectively, are negatively associated (Figs. 29B, D).

Usually taxa are distributed according to their ecological optima and in general, the genera that plotted towards the centre of the diagram are either unrelated to the environmental axes or find their optimum there. In contrast, the taxa arranged towards the borders of the plot show then specializations regarding certain environmental parameters. In the present scenery, *Dinophysis* spp. seemed to prefer higher amounts of PO_4 than *Alexandrium* spp. or *Pseudo-nitzschia* spp. (Figs. 29A, C). Furthermore, the recurrent correlation of *Alexandrium* spp. with the salinity is noteworthy. But in the present analysis these relations should be considered with caution as long as this analysis is done on genus level only and species even from the same family are known to show species specific environmental preferences. In addition to the genera, the toxin groups, obtained from the ELISA

tests of mussel and SPATT samples in 2011 as well as from mussel samples only in 2012, tended to be distributed along the first CCA axis in most cases (Figs. 29A, B, D). Thus, for example, ASP and PSP from mussel samples were positively correlated with the first CCA axis at Miðfjörður in 2012, in strong association with temperature and day length.

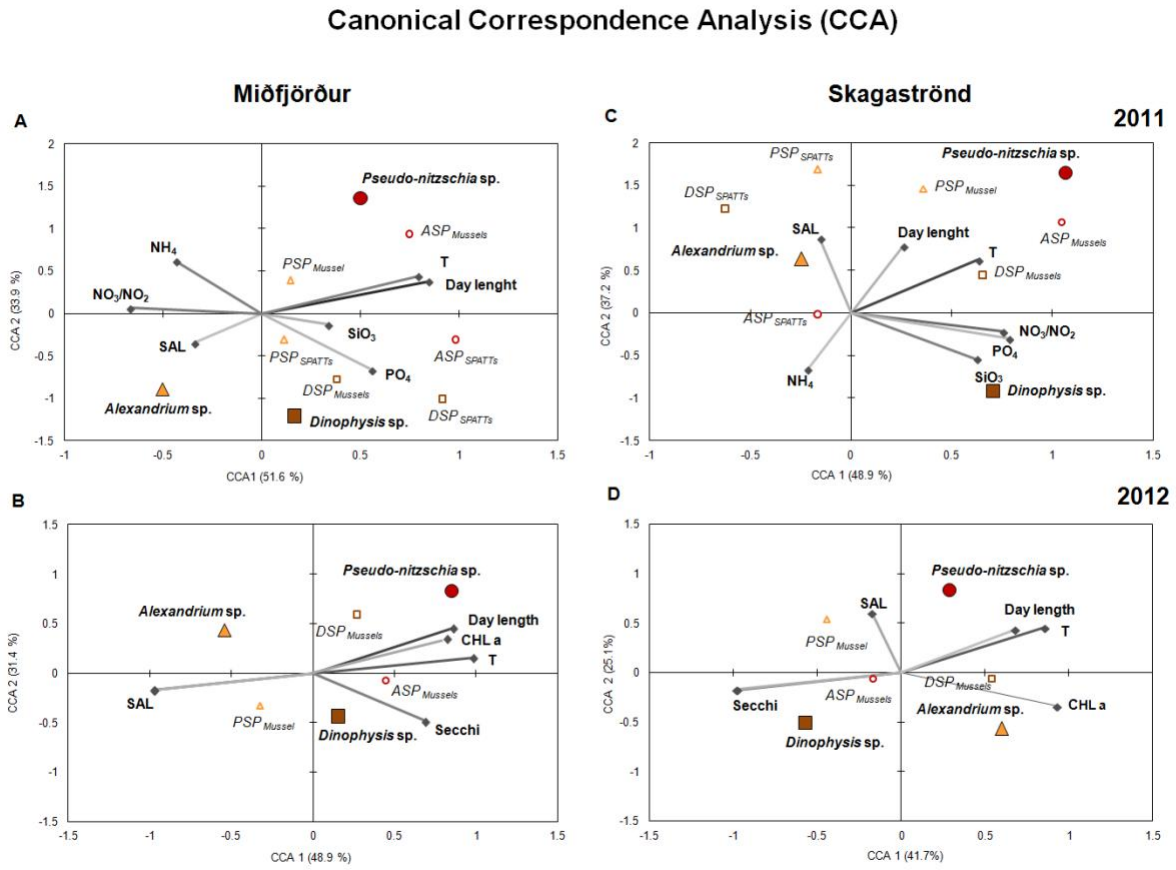


Figure 29. CCA of given environmental variables (■), three toxin groups (△ PSP, □ DSP and ○ ASP) and three potential toxin producing taxa (△*Alexandrium* spp., ■ *Dinophysis* spp. and ● *Pseudo-nitzschia* spp.). Data were obtained from the monitoring at Miðfjörður and Skagaströnd in 2011 and 2012(all variables were multiplied by two). A pre selection of variables was performed by principal component analysis (PCA) for the data of 2011, indicating the variables chl *a* and Secchi depths as components without statistical weight. Thus they were not considered in the CCA of 2011. PCA was not conducted for the data of 2012, due to the lack of nutrient variables. The percentages of variance accounted for by the components are also given; here the most of the variance is accounted for by the first component in all cases. The lengths of the arrows represent the relative importance of different variables in explaining taxa/toxin distributions, while the angles of the arrows relative to the axes and to other variables indicate the strength of their correlations. Abbreviations: CHL a: chlorophyll a, NO_3: nitrate, NH_4: ammonium, NO_2: nitrite, PO_4: phosphate, SAL: salinity, SiO_3: metasilicate.

Results compendium

- In most cases were the ASP, PSP and DSP toxin concentrations directly positive correlated with co-occurring taxa, but not with the producing genera.
- **From the environmental data** day length and temperature were significantly correlated to the potentially toxin producing taxa.

5. Discussion

5.1 Environmental Characterisation in Relation to Total Abundances and Biomasses

Overall, temperatures from 2011 and 2012 were in between the range of published data from the region, with significant increases of temperatures in 3, 10, and 15 m depths at both stations between 2011 and 2012 (up to 2.4 °C in 3 m depth; $p > 0.05$; Figs. 16, 17). Usually the salinity data from the northern marine region of Iceland shows relatively little changes and range from 34.7 to 35 PSU in deeper areas (depth 50 m; Gislason & Astthorsson 1998, Sarsia 2004). In addition, 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). According to Burrell et al. (2013), it seems to be unclear whether these trends are related in any way to the effects of climate change or, relate to natural cyclic variations such as oscillations to the North Atlantic sub polar gyre (Hátún et al. 2005, 2009). In the latter case, 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). In contrast, due to the salinity data obtained from the years 2011 and 2012 in the present monitoring, both stations have to be characterised as brackish water environments (27.5 to 23.9 and 27.4 to 24.3 PSU i.a. at Miðfjörður and Skagaströnd in 2011 and 2012, respectively, Figs. 16, 17). Both stations are closely located to the coast (Fig. 10). At Miðfjörður, as a fjord, a clear freshwater influence from nearby situated streams can be suggested and even the data from the station near Skagaströnd, which was chosen for comparison as a marine station, showed this very strong freshwater influence. Besides the possible impact of freshwater inputs on temperatures and salinities, this influence seems to be also reflected in the depth variations of the nutrient concentrations (Fig. 18). Generally, the overall macronutrient concentrations obtained in the present study were only 0.9% higher for a planktonic realm in comparison to northern European coastal areas (e.g. Howarth & Marino 2006). Beside the extraordinary high ammonium values, the seasonal development of nitrate and nitrite concentrations followed typical seasonal trends, with higher values in spring and autumn and lower ones during summer. The high ammonium concentrations were non-credible and might be a result of inadequate storage conditions of the samples. In addition, If these high ammonium values would occur in the environment they would be highly toxic for the species (the limiting concentration is 100 pM, Bates et al. 1993). In contrast, the Redfield-Brezezinski ratios obtained from the 3 m depth nutrient concentrations, showed a limitation of phosphate as a result of high nitrogen values several times during the monitoring in 2011, being significant at Miðfjörður ($p > 0.05$; Fig. 19). Generally, the Redfield ratio of 16:1 for the molar ratio of ambient concentrations of dissolved inorganic N to P is widely used to infer which nutrient is likely limit the yield of a phytoplankton population. A ratio <16:1 is taken to indicate N limitation and a ratio >16:1 indicates P limitation. However, as acknowledged by Redfield (1963), the ratio is a general basin wide and seasonal average. Phytoplankton often appears to have ratios that approximate Redfield but can display a wide range of cellular composition. Geider and La Roche (2002) reported particulate N:P ratios in the range of 5-34. According to Klausmeier et al. (2004), changes in the cellular storage of nutrients is one reason for the large variation in nutrient stoichiometry in phytoplankton but this is additional to variability in stoichiometry brought about by changes in structural components (e.g. nucleic acids, proteins and

pigments) for which the range in N:P is 7.1e43.3. Examples of large ranges in cellular composition include an observed C:N ratio of 28 for the eustigmatophyte *Nannochloropsis oculata* (Droop) D.J.Hibberd (Flynn et al. 1993) and C:N:P ratios ranging from 682:66:1 to 88:14:1 for the haptophyte *Pavlova lutheri* (Droop) J.C.Green (Tett et al. 1985). This wide variation was due to different forms of nutrient limitation (N or P) in culture. Similar variability in ratios has been shown in euglenoids, dinoflagellates, chlorophyceans, cryptomonads, diatoms, pelagophytes, and cyanobacteria (Tett et al. 2003). The use of the Redfield ratio to differentiate between N and P limiting conditions has been questioned. Since different species have different cellular requirements, some species may be P limited while others are N limited. Zang & Hu (2011) demonstrated different optimal N:P ratios for a number of phytoplankters depending on the form of the N source. A simple relationship between floristic composition and N:P ratio is therefore insufficient to demonstrate a causal link between them. Davidson et al. (2012) evaluated the evidence linking anthropogenically generated shifts in nutrient ratios to HABs in a number of geographical locations where this link has been proposed (e.g. Phaeocystis blooms in the German Bright). In the past several nutrient scenarios were related to HAB species. For example, Bates et al. (1993) observed that *Pseudo-nitzschia pungens* required a high external supply of inorganic NO^-_3 to produce the toxin domoic acid (DA). This is consistent with DA being an amino acid, hence requiring N for its synthesis. Although a few exceptions exist (Garrison et al. 1992), subsequent laboratory studies have found little evidence of significant DA production in non-nutrient limiting conditions. Domoic acid is produced under conditions of nutrient stress which typically occurs when the nutrient ratio is sufficiently skewed that one nutrient becomes limiting for growth (Davidson et al. 2012). In addition, since higher concentrations of DA were produced under Si compared to P limitation, this suggests that the form of nutrient limitation is an important factor in governing toxicity (Davidson et al. 2012). Fehling et al. (2005) also identified a photoperiod effect on growth and toxicity. Wells et al. (2005) reported a link between domoic acid, iron and copper, suggesting that a complex suite of factors influence DA production. The nutrient ratio hypothesis that anthropogenic perturbation in the nutrient supply ratio can influence the floristic composition of phytoplankton is well established, with the influence of a change in the ratio of inorganic N:Si being particularly clear. There is, however, an important caveat: nutrient ratios are only important when the concentration of one nutrient is low enough to limit growth (Davidson et al. 2012). Moreover, with the exception of some specific events, nutrient ratios in coastal waters change relatively slowly even if this change is anthropogenically driven. Due to the lack of water current measurements in the present study the direct influence of agricultural runoffs on the nutrient compositions in the investigated environments is incapable of proof. Hence, a problem with proving or disproving any link between nutrient ratios and the occurrence of harmful algae, rather than a simple diatom/dinoflagellate shift, is frequently the lack of a sufficiently long time series of nutrients, harmful phytoplankton, and other possibly causative environmental variables.

In ecological studies of aquatic areas, chlorophyll distribution has been regarded as an important index for estimation of phytoplankton mass and capacity of primary production (e.g. Smayda 2004). Chlorophyll *a* (chl *a*) is the primary pigment of interest in monitoring programmes. In northern and North West Icelandic coastal habitats chl *a* data varied between <1.0 and >7.0 mg m^3 in spring to 0.5 and 3.0 mg m^3 in summer 1993 and 1994 (Gislason & Astthorsson 1998, no actual data available). In the present study, chl *a* data ranged between 0.2 to 6.0 mg m^3 in the years 2011 and 2012 (=µg L^{-1}; Fig. 20), with strong seasonal differences between the stations. With the exception of a few correlations, changing chl *a* values did not match with the cell counts. This was not unexpected in view of the rich biodiversity and the large size variation of species participating to the

phytoplankton community, in addition to the direct relationship of the physiological status of cells to pigment concentrations (e.g. Stolte et al. 2000). In this context, the increase chl *a* concentrations in 15 m depth, which exceeded several times these of 3 m depth at both stations in 2011 and 2012, is noteworthy (37.5% of the total analysed samples, Fig. 20). The comparison of the Secchi depth showed only at three dates the extension of the euphotic zone to 15 m depth and ranged usually between 4 and 6 m (Figs. 16, 17), suggesting that the high chl *a* values measured in this depth might derived from dead cells. Besides chl *a*, several other pigments and degradation products such as phaeophytin (the magnesium free derivative of chl *a*) may be found at any one time in a given sample (e.g. Brito et al. 2009). Due to spectral overlap between chlorophylls, carotenoids and degradation products, under or over estimation of chl *a* can occur. In the present study, chl *a* was not corrected for its differences in the phaeophytin concentrations and in the counts cells were also not differentiated between live and dead ones (e.g. by using auto fluorescence of the chloroplasts). Thus a further separation between live and dead cell biomass in the depth of 10 and 15 m is not feasible.

Total abundances of the three genera (sum of *Alexandrium* spp., *Dinophysis* spp. and *Pseudo-nitzschia* spp. counts) were found to be closely related to the temperatures obtained during the monitoring in 2011 and 2012 at both stations (Fig. 20). While *Pseudo-nitzschia* showed strong positive correlations to the recorded temperature data, *Alexandrium* spp. and *Dinophysis* spp. were negative or only weak correlated, suggesting that other factors were more important for their growth. In this context, it has to be mentioned that it was not possible to stabilise and grow the collected *Alexandrium tamarense* cysts in the laboratory in Skagaströnd, due to the high temperatures (18-23 °C). The same problem aroused for the culture of the brought stains from Scotland – they were adapted to 15 °C and encysted after a short growth term in the laboratory in Iceland. Generally, growth and culture of cold water adapted species depends besides optimal media conditions from ambient temperatures. Several species which are growing in the environment at temperatures of up to 12 °C can be adapted in the laboratory to higher temperatures (18 °C). But this is a long-term process and requires low temperatures at the start of the cultivation. Regarding the total abundances from the cell counts, the usual seasonal trend in phytoplankton with high spring and autumn abundances as well as significantly lower summer abundances were only partial reflected in the presented data, suggesting the presence of other most abundant species during the seasons. Although nutrient concentrations showed a typical seasonal pattern, evidence of a direct relation of total cell counts of the three genera to the macronutrient concentrations is not given ($p < 0.05$). Results of the CCA showed only for *Dinophysis* spp. a positive correlation to the some of the nutrients during the monitoring in 2011 at both stations (Fig. 29). Overall, the comparison of averaged cell numbers obtained in the present investigation to a study conducted in the northwest part of Iceland in 1994 (Thordardóttir & Eydal 1994) as well as for *Alexandrium* counts in the areas of Breidafjordur and Eyjafjordur (Burrell et al. 2013) showed no significantly differences in cell numbers to these studies ($p < 0.05$).

Conclusions

- Due to our results, the investigated stations are not considerable as marine environments; they have to be regarded as **brackish water habitats**.
- **The macronutrient data from 2011 followed typical seasonal trends**, with higher values in spring and autumn and lower ones during summer, although they were overall higher (up to

0.9%) than expected in a marine planktonic realm in northern European coastal areas. An anthropogenic involvement can be not excluded.

- As expected, the **chl *a* concentrations** showed, except of two events in 2011 and 2012, no positive correlation to the cell counts of *Alexandrium* spp., *Dinophysis* spp. and *Pseudo-nitzschia* spp. Furthermore, the extraordinary high chl *a* values in 15 m might not be related to active cells.
- **Cell counts** of *Alexandrium* spp., *Dinophysis* spp. and *Pseudo-nitzschia* spp. were within the ranges found in other previous Icelandic studies.
- Due to the high temperatures in the laboratory in Skagaströnd, it was not possible to stabilise the isolated cysts as well as the bought strains under culture conditions.

5.2 Correlations of Biotoxin Occurrences in Relation to Potential Toxin Producing Taxa

During the monitoring of *Pseudo-nitzschia* spp., *Dinophysis* spp. and *Alexandrium* spp. in 2011 and 2012 only in the case of the latter taxon a clear relationship of high abundances to higher toxin concentrations were recorded; in all other cases no positive correlations were observed ($p < 0.05$). The positive significant correlations between high cell abundances of *Alexandrium* spp. and higher PSP toxin concentrations were only found at two sampling dates, whereas the highest PSP concentration in August 2011 was not related to the highest *Alexandrium* cell numbers (Figs. 27C, D). Similar observations were made by Burrell et al. (2013). They detected during an investigation of two fjords in west (Breidafjordur) and north (Eyjafjordur) Iceland in 2009, that although the *Alexandrium* cell counts found in Breidafjordur 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 (Burrell et al. 2013).

In the CCA, correlations gave evidence on direct relationships between toxin production and competitive species in several cases. Generally, numerous species are known to occur in high abundances in phytoplanktonic communities (e.g. Sommer 1986, Huisman & Weissing 1999, Hillebrand & Matthiesen 2009). In the present study, species such as *Cylindrotheca* Rabenhorst or *Thalassiosira* Cleve were also frequently observed and noted as present but were not further quantified during the monitoring 2011 and 2012. Generally, competition between species for e.g. light and nutrients is one of the most important features in aquatic systems and the release of bioactive substances toxins by several species to gain certain advantages over other community members is a well known phenomenon (e.g. Mohamed 2002, Legrand et al. 2003, Fistarol et al. 2004, Uronen et al. 2007). So phytoplankton species produce and excrete chemical substances that are affecting other microorganisms in their direct environment - these substances are referred to as allelochemicals (e.g. Granéli 2006) and comprise beside others also hydrophilic and lipophilic phycotoxins (ASP, PSP, DSP). For example, the effect of *Alexandrium* spp. on a natural phytoplankton community was tested by Fistarol et al. (2004) in Hopavågen Bay, Trondheimsfjord, Norway. The authors found that, *A. tamarense* affected the whole phytoplankton community by decreasing the growth rate and changing the community structure (relative abundance of each species, dominant

species). A negative effect of *A. tamarense* was also observed on ciliates, but not on bacteria numbers. In the bioassay with algal monocultures, the diatom *Thalassiosira weissflogii* (Grunow) G.Fryxell & Hasle and the cryptophyte *Rhodomonas* Karsten were exposed to the filtrate of *Alexandrium* spp. All tested *Alexandrium* strains negatively affected *T. weissflogii* and *Rhodomonas* sp. cultures, independent of whether PSP toxins were produced. The compounds released by *Alexandrium* caused lysis of natural and cultured algal cells, suggesting that the allelopathic effect may be connected with previously described ichthyotoxic and haemolytic properties of *Alexandrium* (Fistarol et al. 2004).

Besides allelopathic effects, the release of inside the cells accumulated toxins into the water column after cell death was found in several cases in cyanobacteria blooms (e.g. *Microcystis aeruginosa* (Kützing) Kützing, *Nodularia spumigena* Mertens ex Bornet & Flahault) and other toxins such as the hepatotoxins microcystin and nodularin are also known to give positive reactions with the DSP PP2A test (e.g. Serres et al. 2000). On the other hand, especially in the case of *Alexandrium tamarense* the occurrence of different ribotypes is also discussed. For example, Touzet et al. (2010) demonstrated during August 2007 within two fjordic sea lochs in the Shetland Isles, Scotland, the co-occurrence in the water column of the non-toxic West European (W.E. or Gr. III) and the neurotoxic North American (N.A. or Gr. I) ribotypes of *A. tamarense*, using fluorescent *in situ* hybridisation. In this context, the negative correlation of high *Alexandrium* cell numbers and PSP toxin production in 2012 suggest also the co-occurrence of the non-toxic ribotypes (Gr. III). Here, the method described by Touzet et al. (2010), used in combination with the methanol preserved plankton samples from 2012, could give details about the presence of this ribotypes in northern Icelandic coastal waters.

All these referred examples show possible explanation approaches for the toxin distribution patterns observed in the present investigation, but however, the environmental data of the present study do not provide evidence on the causative effects leading to the general occurrence and composition of the potential toxin producing taxa and the accumulation of ASP, DSP and PSP toxin groups. However, the general explanation for the delay in toxin occurrence relative to the preceded bloom events seems to be that the toxins are synthesised when biomass synthesis slows. Such findings might imply that cells are likely to become more toxic towards the end of a bloom (Davidson et al. 2012). In the present study, 90.6 % of the ASP, DSP and PSP toxin occurrences were positively correlated with decreasing cell numbers after a bloom of *Pseudo-nitzschia* spp., *Dinophysis* spp. and *Alexandrium* spp. Only in one case, regarding DSP and the cell numbers of Dinophysis sp., the toxin occurred before the taxon reached its highest abundances (Fig. 23B). In this context, regarding the predictably of such toxin events, the use of cell counts is still a functional tool for a bloom monitoring. Although high abundances of the taxa do not imply automatically high toxicity and as also found in the present study, high abundances do not precede always higher toxicities; it is at least an indication of the possibility and should be combined with a toxicity screening of the samples. While the regulatory thresholds of phytoplankton in Scotland are 50 x 10^3 cells L^{-1} *Pseudo-nitzschia* spp., 100 cells L^{-1} *Dinophysis* spp. and the presence of *Alexandrium* spp. (http://www.scotland.gov.uk/Publications/2011/03/16182005/37, although in practice ~ 300 cells L^{-1}, which is a more pragmatic value, personal com. Keith Davidson). In contrast, in Icelandic marine habitats the official cell numbers for e.g. *Pseudo-nitzschia pseudodelicatissima* are four times higher (200 x 10^3 cells L^{-1}, but it is differentiated into different species with distinctions in thresholds), for *Dinophysis* spp. five times higher (500 cells L^{-1}) and for *Alexandrium* spp. five times lower (20 cells L^{-1}, http://www.hafro.is/ voktun/vidmid.htm). Besides the fact that the species complex of *Pseudo-nitzschia* spp. can only be determined by the use of electron microscopy as mentioned in the

introduction (Icelandic samples are send to Denmark for analysis), and, however, several species and strains of this complex are able to produce DA and it´s equivalents, it might be useful to comprise these by the term *Pseudo-nitzschia*. Finally, due to the results of the present study threshold recommendations would be 8-10 x 10^3 cells L^{-1} *Pseudo-nitzschia* spp., 80 cells L^{-1} *Dinophysis* spp. and 5 cells L^{-1} *Alexandrium* spp. Additionally, regarding the intervals of cell counting it is recommended to conduct weekly analysis of the abundances at least during the warmer seasons, due to short-term variability of the phytoplankton community. Finally, due to this variability and the complexity of the environmental conditions, future long-term monitoring programs should also include micronutrient data as well as accompanying taxa in order to gain an overview about the environment.

Conclusions

- **Several causes are supposable** for the observed delay in toxin occurrence relative to the preceded bloom events found in the present study. From these, allelopathic interactions as well as the co-occurrence of non-toxin producing strains are presumable.
- Besides the use of molecular-taxonomic methods (e.g. FISH), the most important instrument in the monitoring is the use of **cell counts**, although the predictably of toxin events is restricted. Thus monitoring programs should include also a **toxin pre-screening** of samples.
- Due to the results of the present study, **thresholds** of 8-10 x 10^3 cells L^{-1} *Pseudo-nitzschia* spp., 80 cells L^{-1} *Dinophysis* spp. and 5 cells L^{-1} *Alexandrium* spp. are recommended. As long as, in the latter case the quantification of <20 cells L^{-1} using the Utermöhl technique is not possible, the Scottish model should be used.
- Finally, future **long-term monitoring programs** are needed to comprehend the variability of the environment and to obtain a complete picture of the ecological complexity, considering besides the in the present study used abiotic parameter, also micronutrients and accompanying taxa.

5.3 Biotoxin Compositions and Sensitivity of the Commercial Toxin Tests in Relation to LC Analysis

As outlined in the introduction, DSP toxins are classified into three groups of related polyether compounds: the acidic compound okadaic acid (OA) and its derivatives named Dinophysistoxins (DTX-1,2); the neutral polyetherlactones of the pectenotoxin group PTX-1,2,3,6; yessotoxin (YTX) and its analogue 45-hydroxyyessotoxin (45-OH YTX) (Yasumoto et al. 1985). While DTX-3 is a complex mixture of 7-O-acyl derivatives of OA and DTX-1,2, produced by bioconversion in the digestive glands of shellfish, PTX-2SAs are closely related to PTX-2, containing an open chain carboxylic acid instead of a lactone ring. Under the influence of the metabolic processes, it may through chain closure adopt the structure exerting toxicity. In the present study, PTX-2SAs were found in SPATT and mussel flesh samples. Although concentrations in SPATT bags were significantly lower as compare to the mussel samples the results indicate the presence of PTX-2SAs directly in phytoplankton. In addition to the usual DSP toxins, spirolides (SPX) were also found in higher concentrations in SPATT and mussel

samples. SPX belong to the cyclic imines (CI), which are lipophilic compounds and accumulate in the hepatopancreas of bivalve molluscs. The CI family comprises besides SPXs, gymnodimines (GYMs), pinnatoxins (PnTXs), pteriatoxins (PtTXs), prorocentro lides and spiroprorocentrimines. SPXs, which have a molecular weight of approximately 700 Da (Cembella & Kroc 2008), are mainly produced by the marine dinoflagellate *Alexandrium ostenfeldii/peruvianum* (Cembella et al. 2000, Franco 2006, Pigozzi et al. 2008). They are the largest CI group and they are categorized in eight major groups, which are SPX A, B, C, D, E, F, G and 20-Me-G, in addition to two desmethyl derivatives (Cembella & Krock 2008). These are SPX 13-desMeC, which is derived from SPX C, and SPX 13-desMeD, which originates from SPX D (Ciminiello et al. 2006), whereas details of our unknown 20-Me-G SPX are not published jet and could be subject of future proposals. In this context, the relatively high concentrations of azaspiracids (AZAs) in SPATT samples and of AZA3 in the mussel samples in 2011 should be further discussed. Azaspiracids have recently been identified as the toxins responsible for a series of human intoxications in Europe since 1995, following the consumption of cultured mussels (*Mytilus edulis*) from the west coast of Ireland. James et al. (2002) conducted a comparison to Norwegian mussel samples and found that AZA1 was the predominant toxin and toxin profiles were similar to those found in contaminated Irish shellfish. This was the first report of the occurrence of these azaspiracids outside Ireland and stated that these toxins may occur in shellfish throughout northern Europe. In meantime, several other investigators reported the occurrence of AZAs worldwide (e.g. Ninčević-Gladan et al. 2008, Trainer 2013), but for the Icelandic habitat the results of the present study are the first report. While the Jellett® test results showed not always a correlation to the obtained DSP toxin concentration (in average 41% of the samples), a good accordance of the PP2A OKA® test to the toxin levels confirmed by LC-MS was observed (Fig. 23). Although PP2A method for DSP toxin detection used in the present study showed excellent correlations in relation to the OA and DTXs detection, it can only be recommended as pre-screening in consideration of the general heterogenic nature of the DSP toxin group. Further analysis and comparisons are necessary, using at least triplicate measurements, to verify the reproducibility of test results, which is one of the advantages of LC-MS/MS analyses. Thus, LC-MS/MS methods are considered the methods of choice for cyclic imines analysis in shellfish (EFSA 2010). Several publications have reported LC-MS/MS methods for SPXs, GYMs, PnTXs and PtTXs (e.g. Villar González et al. 2006, Fux et al. 2007, Gerssen et al. 2009a, 2009b, Miles et al. 2010, Selwood et al. 2010). LC-MS/MS methods can provide a LOD of 0.8 µg/kg shellfish meat for 13-desmethyl SPX C, 3.7 µg/kg shellfish meat for GYM A (Gerssen et al. 2009b) and 5 µg/kg shellfish for PnTX G (Miles et al. 2010). In summary, LC-MS/MS is very fast, very specific and it has a detection limit for cyclic imines lower than any other method. Moreover, reference materials for most of the analogues of SPXs, GYMs, PnTXs and PtTXs are lacking (EFSA 2010) and LC-MS/MS methods can identify and accurately quantify only toxins for which standards are available (Campbell et al. 2010). Therefore, it cannot detect all CI-analogues or other emerging toxins. Nevertheless, certain interlaboratory validations have successfully been undertaken and have enabled the EU to appoint LC-MS/MS as the reference method for the detection of lipophilic marine toxins (EU 2011, These et al. 2011).

The LC-FLD method has been proven as a valuable tool in the qualitative and quantitative determination of PSP toxins in shellfish (e.g. Turner et al. 2009). In the present study, a high correlation of the Jellett® PSP tests with the occurrence of PSP toxins was indicated, whereas the cELISA failed in the comparison to the LC method (Fig. 28). Generally, the PSP toxin profiles determined in the present study are similar to those found in other areas where *Alexandrium* spp. predominates such as the UK (Turrel et al. 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 et al. 1998). 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 et al. 2002, Persich et al. 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 et al. 1992). The absence of the N-sulfocarbamoyl toxins C-1,2 from mussel samples taken from both stations, 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 et al. 2007).

Finally the ASP toxin domoic acid (DA) was analysed for its presence and correlation to HPLC analysis in SPATT and mussel samples (Fig. 21), without including the isomers due to the lack of standards. Here the Jellett® tests were all negative in 2012 and invalid in 2011. While, there is no evidence why the test were invalid in 2011, the negative test results might be due to the low DA concentrations in the samples. In general, the ASP Biosense® enzyme ELISA gave excellent results in comparison to the LC analysis for the shell fish samples in 2011, whereas the accordance for the samples in 2012 were significantly weaker. Here the fact that the samples were not immediately after extraction analysed by LC might be the determining factor for the detected differences in DA concentrations.

Conclusions

- For the **ASP toxins (DA equivalents) the ASP Biosense® ELISA** gave excellent results in comparison to the LC analysis for the shell fish samples in 2011, while Jellett® ASP tests did not function.
- In the analysis of **PSP toxins** using the Saxitoxin equivalents the best results were obtained with the Jellett® PSP test, giving a good accordance with the PSP ELISA. In contrast, the comparison to the LC analysis showed only weak correlations.
- The Oka® **DSP test (based on the enzyme PP2A)** showed also good results in the comparison to the LC analysis using OA equivalents, while the while Jellett® DSP tests reflected not always the higher toxin concentrations.
- **Due to the LC results**, which showed the full scope of toxins accumulated by phytoplanktonic organisms, all commercial tests used in the present investigation can only be used as pre-screening methods, due to the fact that for example AZAs and other equivalents are not covered with these methods.
- Future scientific long-term monitoring programs should include **SPATT bags**, due to their extraordinary sensitivity to DSP toxin groups. Furthermore, the LC results of the present study showed also the attendance of *Alexandrium oestenfeldii* in the samples, detected by the SPX fraction in SPATT and mussel samples.
- Finally, **further investigations** are needed to elucidate the identity of the unknown Spirolide, which was found during the investigation in 2011.

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8. Appendix

2011 Miðfjörður – Temperature (T), Salinity and Chlorophyll *a* (Chl *a*) concentrations in different depths.

Sampling Date	T = 1 m [°C]	T = 3 m [°C]	T = 10 m [°C]	T = 15 m [°C]	Salinity = 1 m [PSU]	Salinity = 3m [PSU]	Salinity = 10 m [PSU]	Salinity = 15 m [PSU]	Secchi depths [m]	Chl a 3 m [µg/g]	Chl a 10 m [µg/g]	Chl a 15 m [µg/g]
29.03.11	1.2	1.2	1.3	1.3	28.7	28.7	29.6	29.7	15	–	–	–
19.04.11	2.9***	2.9	3.2	3.1	27.8	27.8	28.0***	27.9	–	0.42	0.36	0.79
06.05.11	4.8	4.8	4.1***	4.0	30.1	30.1	30.7	30.6	4	1.46***	1.27**	1.46*
13.05.11	5.9*	5.9**	5.3	5.0	22.0***	22.0***	24.7**	24.6*	–	5.13*	3.44**	1.57***
25.05.11	5.2**	5.2	4.9***	4.9	27.8	27.8	26.2	25.7**	6.5	1.85*	1.32**	0.96
03.06.11	6.4	6.4	6.2	5.6	29.4	29.4	29.1	29.1	–	1.73*	1.62*	1.68**
10.06.11	–	6.5	6.2**	5.8*	23.5***	26.2*	27.0	27.5**	–	1.09**	1.27**	0.84
22.06.11	–	7.6	6.9	6.7	29.4	29.3	28.0	28.4	7	0.90	0.96	1.38***
30.06.11	8.2	8.1	7.5	7.3***	29.6	25.4*	26.4	26.7	6	0.60	1.02***	1.20***
06.07.11	8.7	8.7	7.6*	7.6	28.1	29.0	26.8	26.3	7	1.08***	0.96	1.80**
13.07.11	9.9	9.7	9.0	8.8	25.3	29.0	26.3	25.7	8	0.42	0.95	0.83
21.07.11	10.2	10.0	9.7	9.1	25.8	26.8	26.0	25.2	9	0.65	0.83	1.31***
28.07.11	9.9	9.9	9.2	8.6	28.3**	25.0**	24.5*	24.2***	7	0.60	0.66	1.37***
04.08.11	10.9	10.9**	9.7	9.5	25.7	25.7*	25.2**	24.0*	4.5	1.49*	1.01**	1.01
09.08.11	10.3***	10.3	10.2*	9.8**	29.0	27.6	26.3	26.0	6	1.26**	1.98*	1.09**
19.08.11	10.2	10.2	9.7	9.5	25.2	25.3**	24.7*	24.5**	5	1.73	1.32*	0.84
25.08.11	10.0	10.0	10.0	9.9	29.3*	28.3	27.4	27.0	6	1.33	0.96	0.54
01.09.11	10.4	10.4	10.1	10.0	28.7	25.1*	24.9	24.9	5	1.87**	1.14	1.02
09.09.11	8.8	8.8	9.2	9.2	27.8	28.9	28.3	28.0	4	2.16*	2.84**	1.50***
∑	133.9	147.5	140	135.7	521.5	517.4	510.1	506	100	25.77	23.91	21.19
Mean	7.88	7.76**	7.37**	7.14*	27.5*	27.2**	26.9**	26.6*	6.67*	1.43***	1.33	1.18

***, p = 0.001, **, p < 0.0001, ***, p > 0.001, [ ], not significant, –, no data available (no sample and/or no measurement, respectively).** Data were compared among themselves and to the data from Skagaströnd 2011 as well as Skagaströnd and Miðfjörður 2012, using one way ANOVA and Duncan's post-hoc test.

2011 Miðfjörður – Macronutrient concentrations in 3 m, 10 m and 15 m depth.

Nutrients	Ammoinium [µM]			Nitrate/nitrite [µM]			Phosphate [µM]			Silicate [µM]		
Sampling Date	3 m	10 m	15 m	3 m	10 m	15 m	3 m	10 m	15 m	3 m	10 m	15 m
29.03.11	3.89**	4.18***	4.04*	7.18*	7.69**	8.01**	0.42***	0.45*	0.45**	5.91*	5.46*	5.13*
19.04.11	1.53	1.38	4.37	5.47*	6.38*	7.22*	0.37***	0.39**	0.42**	5.89*	4.30*	4.34
06.05.11	1.77	1.40	**100.9**	0.13	3.36*	7.10**	0.16	0.26***	0.66*	2.29**	2.37**	3.96
13.05.11	3.14**	2.03*	2.89**	0.21	0.57	1.16**	0.16	0.17***	0.15	6.95*	1.40	1.82***
25.05.11	–	1.56	1.61	–	0.13	0.39	–	0.15	0.15	–	1.82	0.71
03.06.11	1.85	1.04	3.25	0.09	0.02	0.12	0.17	0.13	0.12	1.01**	1.35	1.28*
10.06.11	**36.41**	1.39	1.81	0.35**	0.05	1.58**	0.21**	0.11	0.18***	3.83*	4.09	1.86*
22.06.11	0.86	1.25	1.17	0.01	0.09	0.03	0.10	0.09	0.11	3.03*	3.13	1.02
30.06.11	4.07***	3.06*	4.17**	0.15	0.13	0.17	0.15	0.14	0.16	5.39*	1.07**	0.91
06.07.11	2.08	1.99	2.49*	0.10	0.11	0.13	0.15	0.13	0.13	1.93***	0.66	0.56
13.07.11	2.29	10.46	1.49	0.10	0.70***	0.05	0.12	0.26**	0.10	1.66**	1.20*	0.73
21.07.11	1.81	1.89	2.39	0.05	0.08	0.04	0.11	0.10	0.10	0.97	0.93	0.73
28.07.11	1.71	2.21	2.46	0.08	0.04	0.07	0.10	0.11	0.12	0.93	0.69	0.74
04.08.11	2.09	1.41	1.47	0.07	0.03	0.09	0.16***	0.16***	0.13	1.45***	0.67	0.49
09.08.11	1.67	1.83	2.58	0.03	0.09	0.09	0.13	0.11	0.13***	1.46***	1.03***	1.07**
19.08.11	0.71	2.31*	2.94	0.01	0.13***	0.23***	0.12	0.14	0.18***	2.48**	1.41***	1.59
25.08.11	1.29	0.29	0.40	0.31**	0.21**	0.17***	0.50**	0.20***	0.20**	2.00**	2.33*	2.27*
01.09.11	1.93**	0.18	**66.4**	0.13	0.15	0.68*	0.19***	0.23***	0.47*	2.28*	2.53**	3.08*
09.09.11	0.98	1.17	2.05	0.15***	0.29**	0.27**	0.22**	0.24*	0.26**	4.41*	1.97***	1.51**
∑	33.68	41.02	41.59	14.61	20.44	27.58	3.54	3.56	4.21	53.87	38.39	33.81
Mean	3.89***	2.2**	10.99*	0.8	1.1*	1.45*	0.19	0.19	0.22**	2.9*	2.02**	1.78***

***, p = 0.001, **, p < 0.0001, ***, p > 0.001, [ ], not significant, –, no data available (no sample and/or no measurement, respectively). Data in bold letters were not considered in the statistical analysis.** Data were compared among themselves and to the data from Skagaströnd 2011, using one way ANOVA and Duncan's post-hoc test.

2011 Miðfjörður – Cell counts at 3 m, 10 m and 15 depth

	Pseudo-nitzschia spp. Cells per L^{-1}			*Dinophysis* spp. Cells per L^{-1}			*Alexandrium* spp. Cells per L^{-1}		
Sampling Date	**3 m**	**10 m**	**15 m**	**3 m**	**10 m**	**15 m**	**3 m**	**10 m**	**15 m**
29.03.11	60	0	0	0	0	0	0	0	0
19.04.11	440	260	0	0	0	0	0	0	0
06.05.11	12580**	8580***	4320**	20	0	0	60	0	0
13.05.11	2520	1160	2440	0	0	20	300	160	0
25.05.11	2700***	2440**	680	20	0	20	1880*	760	460
03.06.11	2560	1780	3840	120	60	20	1420*	880	280
10.06.11	2620	4080	10920***	200***	40	0	2620*	2320*	280***
22.06.11	12560*	13360***	16140*	100	140	80	300	920*	660***
30.06.11	3020***	8620	10060	0	20	0	380***	860	900**
06.07.11	4960	4520	3060	0	20	0	400	860***	680
13.07.11	30940***	31340*	18280**	200	220	240**	500	1080*	1480**
21.07.11	12400*	11740**	9520***	180	220	60	1120*	1600**	780***
28.07.11	5480	9140*	5660**	200	240	220	600	240	20
04.08.11	2500	860	480	360**	320***	260***	6500***	1620**	1100*
09.08.11	80	300	220	820*	380***	560**	880**	160	140
19.08.11	20	80	0	1080*	240	40	0	0	40
25.08.11	0	0	60	800*	1020*	660***	0	0	0
01.09.11	0	0	40	780**	1400**	260	0	60	0
09.09.11	460	1500**	1160	2720*	780***	1060*	40	20	0
∑	95900	99760	86880	7600	5100	3500	17000	11540	6820
Mean [x 10^2]	50.5*	52.5***	45.7**	4.0**	2.7***	1.8	8.95***	6.07*	3.59

***, p = 0.001, **, p < 0.0001, ***, p > 0.001, [ ], not significant.** Data were compared among themselves and to the data from Skagaströnd 2011 as well as Skagaströnd and Miðfjörður 2012, using one way ANOVA and Duncan's post-hoc test.

2011 Miðfjörður – ASP toxicity screening								**PSP toxicity screening**	
	SPATT samples				**Mussel samples**			**Mussel samples**	
Sampling Date	**ELISA (BioPol)** DA (ng/g) Dilution *100	**ELISA (BioPol)** DA (ng/g) Dilution *200	**ELISA (MARLAB)** DA (ng/g) Dilution *50	**LC-MS/MS (MARLAB)** DA (ng/g) No Dilution	**ELISA (BioPol)** DA (ng/g)	**ELISA (MARLAB)** DA (ng/g)	**LC-MS/MS (MARLAB)** DA (ng/g)	**ELISA (BioPol)** STX (ng/g)	**HPLC (MARLAB)** STX (ng/g)
29.03.11	–	–	–	–	–	–	–	–	–
19.04.11	–	–	–	–	–	–	–	–	–
06.05.11	–	–	–	–	–	–	–	–	–
13.05.11	–	–	–	–	3.8	4.2	5	0.268*	0.07
25.05.11	0.7	–	0.2	0.2	–	–	–	0.8	–
03.06.11	–	0.1	–	0.2	36.6	28.0	40	0.8	–
10.06.11	–	–	–	–	27.2	24.4***	30	0.8	–
22.06.11	–	0.1	0.4	<LOD	69.8*	50.2**	65*	0.8	4.498*
30.06.11	–	1.4	0.9	<LOD	37.9***	34.2***	35**	0.8	–
06.07.11	–	0.9	0.3	0.6	57.0**	61.1**	65	0.8	–
13.07.11	<LOD	–	1.1**	<LOD	28.1	29.2	30	0.8	–
21.07.11	–	<LOD	0.4	<LOD	43.0	41.2	40	0.8	–
28.07.11	–	2.2	0.6	<LOD	146.7**	123.2*	140*	0.8	–
04.08.11	–	0.4	0.3	<LOD	33.5*	32.6***	30	0.8	4.125**
09.08.11	–	1.9**	–	0.6	9.3	4.8	5	0.8	–
19.08.11	<LOD	–	–	<LOD	5.3	2.1	10	0.8	–
25.08.11	<LOD	1.0	–	<LOD	4.5	1.2	5	0.8	–
01.09.11	<LOD	–	–	<LOD	6.8	5.0	10	0.8	1.681***
09.09.11	0.2	2.7*	–	<LOD	<LOD	1.9	5	0.14	0.07

***, p = 0.001, **, p < 0.0001, ***, p > 0.001, [ ], not significant, –, no data available (no sample and/or no measurement, respectively).** Data were compared among themselves and to the data from Skagaströnd 2011 as well as Skagaströnd and Miðfjörður 2012, using one way ANOVA and Duncan's post-hoc test.

2011 Miðfjörður – DSP toxicity screening – SPATT samples

Non-hydrolysed DSP and LST analysis

Sampling Date	PP2A (BioPol) Total DSP (ng/g)	LC-MS (MARLAB) Total DSP (ng/g)	LC-MS (MARLAB) **PTX2** (ng/g)	LC-MS (MARLAB) **PTX2-sa** (ng/g)	LC-MS (MARLAB) **AZA1** (ng/g)	LC-MS (MARLAB) **AZA-2** (ng/g)	LC-MS (MARLAB) **AZA-3** (ng/g)	LC-MS (MARLAB) **DTX-1** (ng/g)	LC-MS (MARLAB) **DTX-2** (ng/g)	LC-MS (MARLAB) **SPX** des -Me-C (ng/g)	LC-MS (MARLAB) **SPX** 20-Me-G (ng/g)	LC-MS (MARLAB) **SPX** unknown isomer of 20-Me-G (ng/g)
29.03.11	–	–	–	–	–	–	–	–	–	–	–	–
19.04.11	–	–	–	–	–	–	–	–	–	–	–	–
06.05.11	–	–	–	–	–	–	–	–	–	–	–	–
13.05.11	95*	60.8**	28.0	1.1	<LOD	1.8	0.7	18.2***	<LOD	<LOD	25.2	93.0**
25.05.11	<63	38.8***	11.4	0.8	<LOD	1.4	0.6	6.9	<LOD	<LOD	25.5	76.7
03.06.11	<63	12.1	12.5	0.6	<LOD	0.4	0.7	5.0	<LOD	<LOD	52.6**	126.3**
10.06.11	86	41.9	44.6	2.8***	19.4*	2.1	1.0**	15.2	<LOD	<LOD	99.7*	360.9*
22.06.11	81	26.2	44.8***	1.5	<LOD	1.4	1.0	13.2	<LOD	4.2**	81.5**	272.5*
30.06.11	<63	11.6	13.9	1.1	3.7	1.0	0.9	6.9	<LOD	2.6	43.5***	151.9
06.07.11	<63	8.4	14.3	0.5	<LOD	<LOD	0.9	3.7	<LOD	0.8**	18.3	47.1
13.07.11	74	34.3	30.7	1.9	<LOD	<LOD	0.3	20.1*	<LOD	2.1	42.1	152.9**
21.07.11	<63	8.5	6.7	0.4	<LOD	<LOD	0.2	4.6	<LOD	0.3	10.5	29.1
28.07.11	72	18.9	15.5	1.0	<LOD	<LOD	0.7	13.3	<LOD	<LOD	<LOD	<LOD
04.08.11	85***	39.2	31.7	1.6	<LOD	<LOD	0.4	23.8*	<LOD	1.6*	23.6***	69.8***
09.08.11	83***	70.2	77.2**	3.6**	<LOD	<LOD	0.3	39.6*	<LOD	2.0	33.1**	101.6**
19.08.11	<63	40.9	87.0**	2.0	<LOD	<LOD	0.4	23.3**	<LOD	0.5	9.2	25.6
25.08.11	<63	39.7**	39.1	1.6	1.1	<LOD	0.2	11.8	<LOD	<LOD	1.5	<LOD
01.09.11	90**	240.0*	327.5*	10.2*	2.2	<LOD	0.6	74.8	<LOD	<LOD	11.9	23.8
09.09.11	0.2	2.7	–	<LOD	<LOD	1.9	5			<LOD	25.2**	93.0

***, p = 0.001, **, p < 0.0001, ***, p > 0.001, [ ], not significant, –, no data available (no sample and/or no measurement, respectively).** Data were compared among themselves and to the data from Skagaströnd 2011 as well as Skagaströnd and Miðfjörður 2012, using one way ANOVA and Duncan's post-hoc test.

2011 Miðfjörður – DSP toxicity screening – Mussel samples

	Non-hydrolysed DSP and LST analysis											Hydrolysed DSP		
Sampling Date	**LC-MS (MARLAB)** Total DSP (ng/g)	**LC-MS (MARLAB) PTX2** (ng/g)	**LC-MS (MARLAB) PTX2-sa** (ng/g)	**LC-MS (MARLAB) AZA1** (ng/g)	**LC-MS (MARLAB) AZA-2** (ng/g)	**LC-MS (MARLAB) AZA-3** (ng/g)	**LC-MS (MARLAB) DTX-1** (ng/g)	**LC-MS (MARLAB) DTX-2** (ng/g)	**LC-MS (MARLAB) SPX** des -Me-C (ng/g)	**LC-MS (MARLAB) SPX** 20-Me-G (ng/g)	**LC-MS (MARLAB) SPX** unknown isomer of 20-Me-G (ng/g)	**PP2A (BioPol)** Total DSP (ng/g)	**PP2A (MARLAB)** Total DSP (ng/g)	**LC-MS (MARLAB)** Total DSP (ng/g)
29.03.11	–	–	–	–	–	–	–	–	–	–	–	–	–	–
19.04.11	–	–	–	–	–	–	–	–	–	–	–	–	–	–
06.05.11	–	–	–	–	–	–	–	–	–	–	–	–	–	–
13.05.11	0.0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	–	<LOD	<LOD	<LOD	<63	<63	<LOD
25.05.11	–	–	–	–	–	–	–	–	–	–	–	–	–	–
03.06.11	<LOD	<LOD	4.0	<LOD	<LOD	<LOD	<LOD	–	<LOD	8.3**	2.3	<63	<63	<LOD
10.06.11	<LOD	<LOD	7.4	<LOD	<LOD	<LOD	<LOD	–	7.3	8.9*	38.5*	97***	<63	<LOD
22.06.11	<LOD	<LOD	4.4	<LOD	<LOD	<LOD	<LOD	–	<LOD	3.3	19.2***	111	<63	8
30.06.11	5.9	<LOD	3.7	<LOD	1.0	<LOD	<LOD	–	<LOD	7.1	<LOD	<63	<63	<LOD
06.07.11	<LOD	<LOD	4.9	<LOD	<LOD	<LOD	<LOD	–	<LOD	<LOD	10.3	92	<63	<LOD
13.07.11	6.2	<LOD	4.3	<LOD	<LOD	<LOD	<LOD	–	<LOD	<LOD	<LOD	<63	<63	10
21.07.11	<LOD	<LOD	8.3***	<LOD	<LOD	<LOD	<LOD	–	<LOD	<LOD	7.1	<63	<63	10
28.07.11	11.1	<LOD	15.9*	<LOD	<LOD	<LOD	<LOD	–	<LOD	<LOD	<LOD	94	<63	20
04.08.11	<LOD	<LOD	9.1**	<LOD	<LOD	<LOD	<LOD	–	<LOD	<LOD	2.2	78	<63	15
09.08.11	4.8	<LOD	7.0	<LOD	<LOD	<LOD	<LOD	–	<LOD	<LOD	<LOD	91	<63	33***
19.08.11	14.3***	<LOD	4.5	<LOD	<LOD	<LOD	0.0	–	<LOD	<LOD	20.6**	178**	86***	62**
25.08.11	21.9**	<LOD	7.1	<LOD	<LOD	<LOD	6.4	–	<LOD	<LOD	6.9	207**	119*	81**
01.09.11	80.0*	<LOD	7.8***	<LOD	<LOD	<LOD	23.9*	–	<LOD	<LOD	9.8	293*	161**	149*
09.09.11	<LOD	<LOD	<LOD	26.5*	<LOD	<LOD	<LOD	–	<LOD	<LOD	<LOD	<63	<63	14

***, p = 0.001, **, p < 0.0001, ***, p > 0.001, [ ], not significant, –, no data available (no sample and/or no measurement, respectively).** Data were compared among themselves and to the data from Skagaströnd 2011 as well as Skagaströnd and Miðfjörður 2012, using one way ANOVA and Duncan's post-hoc test.

2011 Skagaströnd – Temperature (T), Salinity and Chlorophyll *a* (Chl *a*) concentrations in different depths.

Sampling Date	T = 1 m [°C]	T = 3 m [°C]	T = 10 m [°C]	T = 15 m [°C]	Salinity = 1 m [PSU]	Salinity = 3m [PSU]	Salinity = 10 m [PSU]	Salinity = 15 m [PSU]	Secchi depths [m]	Chl a 3 m [µg/g]	Chl a 10 m [µg/g]	Chl a 15 m [µg/g]
11.05.11	4.3	–	4.2	4.1	30.3	–	28.5	27.8	8	–	–	–
25.05.11	5.1*	–	4.7*	4.7***	24.2***	–	23.0**	22.6**	4	–	–	–
01.06.11	5.1	5.3**	5.1	4.9	29.8	30.0	30.3	30.5	6.5	1.74*	1.80*	1.63*
21.06.11	6.6	6.2	6.0*	5.6	30.8	31.1	31.18	31.4	–	0.85***	1.24**	1.20**
30.06.11	7.2	6.8	6.5	6.3	25.2	25.0**	24.7***	24.5*	–	0.73	0.42	0.35
07.07.11	8.4	7.4	6.9**	6.8*	30.3	29.4	27.9	27.4	10.5	0.90***	–	1.02***
14.07.11	9.2	8.8***	7.8	7.5	27.7	27.7	26.9	26.4	11	–	–	–
22.07.11	10.9**	9.6*	8.3	7.4*	25.7*	25.5***	24.4**	24.0*	9	1.44**	1.18*	1.50**
27.07.11	11.0*	11.0**	10.9***	9.0	26.6	26.2	25.9	24.7**	6	1.08	1.43*	1.67*
09.08.11	–	7.9	8.0*	8.0	23.3*	23.1**	22.6***	22.6*	6	1.31**	1.56**	0.72***
10.08.11	7.9	9.7	8.8	8.7	32.4	30.9	30.9	29.4	7.9	0.98***	1.12***	0.72
18.08.11	9.0**	8.9*	8.8	8.8*	26.5	32.1	31.3	31.1	7	1.19*	1.22	0.90***
25.08.11	8.0	7.9**	7.7**	8.0	28.5	25.4	23.3*	23.2**	8	1.33**	–	1.21**
31.08.11	10.1	9.8	9.4	9.3	23.3**	27.9	27.2	26.6	6	–	–	–
09.09.11	7.9	7.9	8.0	8.0	–	23.1**	22.6	22.6***	6	0.46	0.51**	0.45
∑	110.7	107.2	111.1	107.1	384.6	334.3	400.68	394.8	95.9	12.01	10.48	10.92
Mean	7.91	8.25**	7.41*	7.14	27.5**	25.7***	26.7*	26.3**	7.38	0.92***	0.86	1.01*

***, p = 0.001, **, p < 0.0001, ***, p > 0.001, [ ], not significant, –, no data available (no sample and/or no measurement, respectively).** Data were compared among themselves and to the data from Miðfjörður 2011 as well as Skagaströnd and Miðfjörður 2012, using one way ANOVA and Duncan's post-hoc test.

2011 Skagaströnd – Macronutrient concentrations in 3 m, 10 m and 15 m depth.

Nutrients	Ammoinium [µM]			Nitrate/nitrite [µM]			Phosphate [µM]			Silicate [µM]		
Sampling Date	3 m	10 m	15 m	3 m	10 m	15 m	3 m	10 m	15 m	3 m	10 m	15 m
11.05.11	**37.38**	1.82*	0.44	8.17*	7.49**	6.11**	0.86**	0.46**	0.46**	4.12*	3.54**	2.94**
25.05.11	1.24**	1.23*	2.51	3.21**	6.68*	5.41*	0.26***	0.41**	0.45***	4.70*	4.74*	3.19*
01.06.11	0.17	**34.45**	0.91***	0.38	1.08***	1.68**	0.20	0.34***	0.26**	1.96*	4.02***	2.27**
21.06.11	**38.17**	0.65	0.98***	1.52***	0.42	0.68***	0.47***	0.17	0.19	2.24*	1.55**	1.03***
30.06.11	0.71**	0.84***	1.36*	0.31***	0.45***	0.72***	0.17	0.19	0.19	1.16	0.44	0.90***
07.07.11	0.71**	0.62	0.79***	0.16	0.42	0.39	0.12	0.18	0.18	1.11**	0.27	0.32
14.07.11	0.68	0.74	1.64	0.10	0.12	0.15	0.13	0.14	0.13	1.64*	0.35	0.35
22.07.11	2.29*	0.97***	1.82**	0.15	0.08	0.11	0.13	0.12	0.11	3.40*	0.30	0.92
27.07.11	1.42**	0.88	**25.97**	0.15	0.11	1.66**	0.12	0.14	0.54***	3.48*	2.77	1.24
03.08.11	0.89***	1.96**	**21.97**	0.16	0.12	1.79*	0.13	0.15	0.65**	1.10**	0.32***	1.02
10.08.11	0.39	0.70	1.37**	0.10	0.18	0.45	0.17	0.13	0.19	2.54*	1.81*	1.85*
18.08.11	2.06*	2.11*	2.23*	0.47*	0.44	0.56***	0.30***	0.30	0.24	3.57*	2.53***	2.21*
25.08.11	1.85**	1.40***	1.67**	0.75**	0.74**	1.28**	0.31***	0.29	0.32***	2.26*	2.21**	2.29**
31.08.11	0.98	**50.97**	2.33*	0	3.40*	0.21	0.20	2.29*	0.16	15.23*	3.39**	6.26*
09.09.11	3.96*	3.04**	4.22*	5.85*	4.31*	4.45**	0.87***	0.95*	1.18***	8.13*	4.87*	4.95***
∑	17.39	16.94	22.28	21.48	26.05	25.65	4.45	6.24	5.22	56.66	33.11	31.74
Mean	6.2*	6.8**	4.68***	1.4	1.74*	1.7	0.29***	0.42**	0.35***	3.78*	2.21***	2.12**

***, p = 0.001, **, p < 0.0001, ***, p > 0.001, –, not significant. Data in bold letters were not considered in the statistical analysis.**

Data were compared among themselves and to the data from Miðfjörður 2011, using one way ANOVA and Duncan's post-hoc test.

2011 Skagaströnd – Cell counts at 3 m, 10 m and 15 depth

	Pseudo-nitzschia spp. Cells per L^{-1}			*Dinophysis* spp. Cells per L^{-1}			*Alexandrium* spp. Cells per L^{-1}		
Sampling Date	**3 m**	**10 m**	**15 m**	**3 m**	**10 m**	**15 m**	**3 m**	**10 m**	**15 m**
11.05.11	1680**	800*	460***	0	0	0	0	0	0
25.05.11	1800***	2380*	2120***	20	0	0	100	0	0
01.06.11	15820*	17160***	10320**	60	20	40	260	120	0
21.06.11	23900**	13780*	19060*	60	0	0	300**	260	0
30.06.11	72700***	24160**	18040*	0	0	0	20	0	0
07.07.11	46700**	3720*	220***	20	0	0	20	80	0
14.07.11	11480*	10200**	8500	120**	0	0	1020*	800**	500
22.07.11	38880	60240	291500**	320*	60	80	2320***	520	1200*
27.07.11	30200**	26240***	11920*	280***	60	620**	4560**	2940*	8460***
03.08.11	680*	260***	820*	220**	380*	280***	3240	2140	1040
10.08.11	80	400*	80	60	120	80	0	0	0
18.08.11	3200*	3540**	2460***	20	120	20	0	120	140
25.08.11	30640***	19540**	19940*	180	320**	180	0	0	0
31.08.11	484000*	10380*	737000**	1020*	2080**	580*	0	0	0
09.09.11	920***	1560**	2260***	160**	120	220*	0	0	0
∑	762680	194360	1124700	2540	3280	2100	11840	6980	11340
Mean [x 10^2]	508.45	129.57*	749.8**	1.69***	2.19*	1.4*	7.89*	4.65***	7.56*

***, p = 0.001, **, p < 0.0001, ***, p > 0.001, [ ], not significant.** Data were compared among themselves and to the data from Miðfjörður 2011 as well as Skagaströnd and Miðfjörður 2012, using one way ANOVA and Duncan's post-hoc test.

2011 Skagaströnd – ASP toxicity screening								**PSP toxicity screening**	
	SPATT samples				**Mussel samples**			**Mussel samples**	
Sampling Date	**ELISA (BioPol)** DA (ng/g) Dilution *100	**ELISA (BioPol)** DA (ng/g) Dilution *200	**ELISA (MARLAB)** DA (ng/g) Dilution *50	**LC-MS/MS (MARLAB)** DA (ng/g) No Dilution	**ELISA (BioPol)** DA (ng/g)	**ELISA (MARLAB)** DA (ng/g)	**LC-MS/MS (MARLAB)** DA (ng/g)	**ELISA (BioPol)** STX (ng/g)	**HPLC (MARLAB)** STX (ng/g)
11.05.11	0.1	–	–	<LOD	3.6	<LOD	30	–	–
25.05.11	<LOD	3.9**	–	<LOD	8.4	3.5	20	0.125	0.014
01.06.11	<LOD	–	0.5	0.6	179.6*	137.0**	170*	0.154*	–
21.06.11	1.5	1.8	1.8	1.2	253.1**	234.2*	300*	0.8	–
30.06.11	1.9*	4.1*	2.3***	3.0*	200.1*	171.9**	185***	0.8	–
07.07.11	0.9**	–	0.8	1.0	303.2***	274.3**	275*	0.8	0.317
14.07.11	<LOD	<LOD	–	1.2	1093.2*	527.6**	445*	0.8	–
22.07.11	–	–	–	–	174.0	91.8**	145***	0.8	–
27.07.11	<LOD	1.2	0.4	<LOD	24.9	15.0	25	0.8	–
09.08.11	0.8	–	–	0.2	8.5	<LOD	5	0.8	12.7**
10.08.11	0.5	2.9	–	0.2	13.5	3.9	10	0.8	–
18.08.11	0.1	1.1	–	<LOD	14.9	8.1	15	0.8	–
25.08.11	<LOD	–	–	<LOD	17.2	3.4	10	0.125*	1.844
31.08.11	–	–	–	–	–	–	–	0.8	–
09.09.11	–	–	–	–	–	–	–	0.8	–

***, p = 0.001, **, p < 0.0001, ***, p > 0.001, [ ], not significant, –, no data available (no sample and/or no measurement, respectively).** Data were compared among themselves and to the data from Skagaströnd 2011 as well as Skagaströnd and Miðfjörður 2012, using one way ANOVA and Duncan's post-hoc test.

2011 Skagaströnd – DSP toxicity screening – SPATT samples

Non-hydrolysed DSP and LST analysis

Sampling Date	PP2A (BioPol) Total DSP (ng/g)	LC-MS (MARLAB) Total DSP (ng/g)	LC-MS (MARLAB) **PTX2** (ng/g)	LC-MS (MARLAB) **PTX2-sa** (ng/g)	LC-MS (MARLAB) **AZA1** (ng/g)	LC-MS (MARLAB) **AZA-2** (ng/g)	LC-MS (MARLAB) **AZA-3** (ng/g)	LC-MS (MARLAB) **DTX-1** (ng/g)	LC-MS (MARLAB) **DTX-2** (ng/g)	LC-MS (MARLAB) **SPX** des -Me-C (ng/g)	LC-MS (MARLAB) **SPX** 20-Me-G (ng/g)	LC-MS (MARLAB) **SPX** unknown isomer of 20-Me-G (ng/g)
11.05.11	<63	26.0	2.4	<LOD	<LOD	<LOD	0.4	<LOD	<LOD	–	–	–
25.05.11	<63	37.2	5.0	<LOD	<LOD	<LOD	1.0	<LOD	<LOD	<LOD	16.1	7.5
01.06.11	77***	31.7	10.0	1.2	<LOD	1.2	0.9	<LOD	<LOD	<LOD	77.3*	68.7**
21.06.11	<63	9.4	10.0	1.2	<LOD	1.2	0.9	<LOD	<LOD	<LOD	7.2	25.6
30.06.11	<63	6.9***	6.5	0.6	4.8*	0.6	1.0**	<LOD	<LOD	0.3	38.8***	30.2
07.07.11	<63	5.7	7.3	<LOD	<LOD	<LOD	0.3	<LOD	<LOD	0.3	11.8**	31.4
14.07.11	<63	14.0	27.6	0.9	<LOD	0.9	0.4	<LOD	<LOD	<LOD	28.1	83.9*
22.07.11	<63	6.4	20.5**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.2	25.2	75.2*
27.07.11	98*	22.1	26.8**	1.1	<LOD	1.1	0.3	8.7*	2.0	1.5*	42.9	149.0*
09.08.11	<63	9.2	29.6*	0.9	<LOD	0.9	0.6	1.4	<LOD	0.7	12.9	30.4
10.08.11	76	17.3	39.2*	1.8*	<LOD	1.8**	0.6	5.0	<LOD	0.9**	21.0**	61.0***
18.08.11	<63	12.3	46.1	0.7	<LOD	0.7	0.2	2.6	<LOD	0.6	15.4	38.8
25.08.11	73	23.1**	14.9***	1.4	<LOD	<LOD	0.1	4.9**	<LOD	<LOD	5.5	8.9
31.08.11	–	–	–	–	–	–	–	–	–	–	–	–
09.09.11	–	–	–	–	–	–	–	–	–	–	–	–

***, p = 0.001, **, p < 0.0001, ***, p > 0.001, [ ], not significant, –, no data available (no sample and/or no measurement, respectively).** Data were compared among themselves and to the data from Skagaströnd 2011 as well as Skagaströnd and Miðfjörður 2012, using one way ANOVA and Duncan's post-hoc test.

2011 Skagaströnd – DSP toxicity screening – Mussel samples

	Non-hydrolysed DSP and LST analysis											Hydrolysed DSP		
Sampling Date	**LC-MS (MARLAB)** Total DSP (ng/g)	**LC-MS (MARLAB) PTX2** (ng/g)	**LC-MS (MARLAB) PTX2-sa** (ng/g)	**LC-MS (MARLAB) AZA1** (ng/g)	**LC-MS (MARLAB) AZA-2** (ng/g)	**LC-MS (MARLAB) AZA-3** (ng/g)	**LC-MS (MARLAB) DTX-1** (ng/g)	**LC-MS (MARLAB) DTX-2** (ng/g)	**LC-MS (MARLAB) SPX** des -Me-C (ng/g)	**LC-MS (MARLAB) SPX** 20-Me-G (ng/g)	**LC-MS (MARLAB) SPX** unknown isomer of 20-Me-G (ng/g)	**PP2A (BioPol)** Total DSP (ng/g)	**PP2A (MARLAB)** Total DSP (ng/g)	**LC-MS (MARLAB)** Total DSP (ng/g)
11.05.11	–	–	–	–	–	–	–	–	–	–	–	–	–	–
25.05.11	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	–	<LOD	<LOD	<LOD	<63	<63	<LOD
01.06.11	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	–	<LOD	3.6**	<LOD	80.7**	<63	<LOD
21.06.11	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	–	<LOD	1.3	<LOD	<63	<63	<LOD
30.06.11	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	–	<LOD	<LOD	<LOD	<63	<63	<LOD
07.07.11	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	–	<LOD	<LOD	<LOD	87.4**	<63	<LOD
14.07.11	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	–	<LOD	3.4	<LOD	110.7	<63	<LOD
22.07.11	2.1	<LOD	9.8*	<LOD	1.5	<LOD	<LOD	–	<LOD	5.1	1.4	120.1*	<63	8.7
27.07.11	<LOD	<LOD	7.1*	<LOD	2.3**	<LOD	<LOD	–	<LOD	8.8*	19.6*	139.4*	<63	11.1**
09.08.11	<LOD	<LOD	8.6*	<LOD	<LOD	<LOD	<LOD	–	<LOD	2.8***	1.6	105.9	<63	20.6*
10.08.11	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	–	<LOD	<LOD	<LOD	<63	<63	4.8
18.08.11	<LOD	<LOD	2.0	<LOD	0.5	<LOD	<LOD	–	<LOD	1.2	<LOD	94.5***	<63	11.9
25.08.11	<LOD	<LOD	1.8	<LOD	2.4***	<LOD	<LOD	–	<LOD	<LOD	<LOD	108.6	<63	12.5
31.08.11	<LOD	<LOD	2.7	<LOD	3.2*	<LOD	<LOD	–	<LOD	<LOD	<LOD	114.6	<63	20.9
09.09.11	<LOD	<LOD	4.2***	<LOD	2.1	<LOD	4.8*	–	<LOD	3.4**	<LOD	111.0	73.6*	91.8***

***, p = 0.001, **, p < 0.0001, ***, p > 0.001, [ ], not significant, –, no data available (no sample and/or no measurement, respectively).** Data were compared among themselves and to the data from Skagaströnd 2011 as well as Skagaströnd and Miðfjörður 2012, using one way ANOVA and Duncan's post-hoc test.

2012 Miðfjörður – Temperature (T), Salinity and Chlorophyll *a* (Chl *a*) concentrations in different depths.

Sampling Date	T = 1 m [°C]	T = 3 m [°C]	T = 10 m [°C]	T = 15 m [°C]	Salinity = 1 m [PSU]	Salinity = 3m [PSU]	Salinity = 10 m [PSU]	Salinity = 15 m [PSU]	Secchi depths [m]	Chl a 3 m [µg/g]	Chl a 10 m [µg/g]	Chl a 15 m [µg/g]
17.04.12	3.9	3.9	3.2	3.1	8.1*	8.1**	10.7***	10.9*	6.0	2.74 ± 0.51*	0.91 ± 0.15**	0.67 ± 0.08
27.04.12	4.1	4.1**	4.1	4.0	20.0**	–	17.3***	–	10.0	0.44 ± 0.07	0.53 ± 0.08	0.64 ± 0.11
07.05.12	4.5*	4.7	4.1	3.8	–	22.5***	21.4**	20.2*	7.0	1.09 ± 0.18	5.46 ± 0.11*	6.19 ± 0.26***
18.05.12.	5.0	4.9***	4.4*	4.3	23.4*	22.8	22.3	21.8*	10.0**	–	–	–
24.05.12	6.6**	5.0*	4.8***	4.7	19.0*	18.3*	17.4**	18.1***	8.0	0.79 ± 0.08	0.79 ± 0.09	2.31 ± 0.33***
01.06.12	6.7	6.2	5.8**	5.4	21.4***	20.6**	20.1*	19.7***	10.0	0.65 ± 0.09	1.94 ± 0.14*	2.24 ± 0.16**
08.06.12	8.0	7.6	7.4	7.2	23.3**	22.1**	21.7**	21.5*	7.0	1.03 ± 0.09	1.39 ± 0.15**	0.85 ± 0.27*
15.06.12	8.3	7.9	7.2	7.0**	25.2***	25.1	24.3**	22.6	8.0	0.43 ± 0.10	0.48 ± 0.07*	0.26 ± 0.05
21.06.12	9.5*	9.4**	9.3	8.6	28.3	27.6	27.3	27.2	9.0***	0.42 ± 0.08	0.78 ± 0.03	0.73 ± 0.04
29.06.12	11.4	11.4	11.3	11.1**	27.6	27.0	26.2	26.0	6.0	1.52 ± 0.12**	1.82 ± 0.15*	1.83 ± 0.11*
05.07.12	12.6	12.4	10.7	7.7*	26.2	25.6*	25.0***	24.4	8.5	1.08 ± 0.09**	1.02 ± 0.08**	1.20 ± 0.26*
12.07.12	13.3	12.5	10.4	10.2	28.4	27.8	26.3	25.8	6.0	1.51 ± 0.11**	2.07 ± 0.13**	1.34 ± 0.14**
19.07.12	12.8	12.7	12.2*	11.6	24.8	24.7	23.8	23.5	4.0	1.67 ± 0.28*	2.28 ± 0.37**	2.54 ± 0.27***
26.07.12	11.7	11.7	11.6	11.7	17.9***	18.3***	18.9**	19.1*	5.0	1.96 ± 0.37**	2.12 ± 0.22**	2.18 ± 0.44***
31.07.12	12.7	12.5*	12.2***	12.1**	23.7*	24.3**	24.7*	24.9***	7.0*	3.62 ± 0.81*	6.70 ± 0.25*	5.55 ± 0.32*
09.08.12	11.0	11.0	10.8	10.7	27.0	26.2	24.8	24.3**	7.0	2.21 ± 0.54	5.06 ± 0.16*	4.21 ± 0.25*
15.08.12	10.5*	10.5	10.1	9.9	27.5	26.4	25.7	25.4	5.0	1.41 ± 0.17	1.34 ± 0.14*	0.61 ± 0.08
24.08.12	11.0	11.0	11.0	11.0	27.4	26.8	24.1	25.6	4.0	2.67 ± 0.76*	2.59 ± 0.22**	2.34 ± 0.13*
29.08.12	10.3	10.2	10.2	10.1	28.4	27.6	24.7	24.0	4.0	1.45 ± 0.25	1.41 ± 0.16*	1.31 ± 0.14*
05.09.12	9.9	9.9	9.8	9.8	26.9	26.5	26.7	26.5	4.0	2.00 ± 0.21*	0.98 ± 0.09	0.99 ± 0.13
14.09.12	8.5	8.9	9.1	9.2	25.2**	23.4***	22.9**	22.8***	4.0	0.99 ± 0.10**	0.90 ± 0.10	1.09 ± 0.07
19.09.12	8.7	8.8	9.0	9.0	24.6	24.3	24.0	23.7	10.0***	0.61 ± 0.09	0.39 ± 0.05	0.36 ± 0.02
28.09.12	8.3	8.3	8.3**	8.3*	24.5	23.5**	22.2***	22.0**	6.0	4.84 ± 0.59	4.27 ± 0.22	5.26 ± 0.44***
∑	209.3	205.5	197	190.5	528.8	519.5	522.5	500	155.5	35.1	45.2	44.71
Mean	9.1	8.9***	8.6**	8.3**	24.0	23.6**	22.7*	22.7***	6.7*	1.6***	2.05**	2.03*

***, p = 0.001, **, p < 0.0001, ***, p > 0.001, [ ], not significant, –, no data available (no sample and/or no measurement, respectively).** Data were compared to the data from Skagaströnd 2012 as well as Skagaströnd and Miðfjörður 2011, using one way ANOVA and Duncan's post-hoc test. For Chl *a* concentrations mean values ± SD are included from triplicate measurements.

2012 Miðfjörður – Cell counts at 3 m, 10 m and 15 depth

	Pseudo-nitzschia spp. Cells per L^{-1}			*Dinophysis* spp. Cells per L^{-1}			*Alexandrium* spp. Cells per L^{-1}		
Sampling Date	**3 m**	**10 m**	**15 m**	**3 m**	**10 m**	**15 m**	**3 m**	**10 m**	**15 m**
17.04.12	5200*	1700**	1480***	0	0	0	0	0	0
27.04.12	0	80	120	0	80	0	60	20	0
07.05.12	1500*	400	540	0	20	20	60	100	0
18.05.12.	1140*	1240	1440*	40	0	0	260*	20	20
24.05.12	480***	1400**	1080*	20	20	20	40	360***	200**
01.06.12	2620**	2100*	1300***	0	40	0	140	60	40
08.06.12	1180*	500***	940*	160***	300*	60	660	1540*	580*
15.06.12	2200**	960***	1200*	1040**	1260*	1060***	380	1820*	1720*
21.06.12	4840***	1660**	6220***	2180***	1800*	840***	1060***	1560**	2920***
29.06.12	0	0	0	2940*	2660*	3160**	1380*	1720**	1860*
05.07.12	0	80	40	4080**	2380***	2120*	1340***	400***	80
12.07.12	440***	180	1140	2460*	2660**	2560***	960**	460***	400**
19.07.12	200	160	240	760***	1580*	2180***	600**	380*	0
26.07.12	0	0	0	3900**	8560**	7640*	0	0	0
31.07.12	0	0	0	2580**	3020***	5960**	0	0	0
09.08.12	0	0	0	1440*	3240**	1760*	0	0	20
15.08.12	3000***	8840*	6360**	500***	1660	1180*	0	0	0
24.08.12	600	520	500	480*	820**	160***	0	0	0
29.08.12	260	20	60	80	60	120	0	0	0
05.09.12	360	80	100	180	100	60	0	0	0
14.09.12	1220*	200***	160	60	40	40	0	0	0
19.09.12	60	0	40	0	100	20	0	0	0
28.09.12	4440***	11700**	3820***	20	20	100*	0	0	0
∑	29740	31820	26780	22920	30420	29060	6940	8440	7840
Mean [x 10^2]	12.93**	13.84***	11.64*	9.97*	13.23***	12.63*	3.02	3.67***	3.41**

***, p = 0.001, **, p < 0.0001, ***, p > 0.001, [ ], not significant.** Data were compared to the data from Skagaströnd 2012 as well as Skagaströnd and Miðfjörður 2011, using one way ANOVA and Duncan's post-hoc test.

2012 Miðfjörður – Toxicity screening – Mussel samples only

	ASP		DSP			PSP	
Sampling Date	**ELISA (BioPol)** DA (ng/g)	**LC-MS/MS (MARLAB)** DA (ng/g)	**PP2A (BioPol)** Total DSP-H (ng/g)	**LC-MS (MARLAB)** Total DSP-H (ng/g)	**LC-MS (MARLAB)** Total DSP-nH (ng/g)	**ELISA (BioPol)** STX (ng/g)	**HPLC (MARLAB)** STX (ng/g)
17.04.12	1.7	–	<63	–	–	–	–
27.04.12	5.9***	–	43.7	–	–	0.8	0.339***
07.05.12	0.4	–	<63	0	7.7	0.07	–
18.05.12.	5.4**	4	<63	–	–	0.154**	–
24.05.12	<LOD	–	151.5**	–	–	0.605***	–
01.06.12	1.3***	–	<63	–	–	0.588*	–
08.06.12	<LOD	–	<63	–	–	0.138***	–
15.06.12	0.5	–	59.8	–	–	0.8	0.913***
21.06.12	<LOD	–	129.3**	95.5	163.3***	0.629**	–
29.06.12	–	–	152.2	–	–	0.8	–
05.07.12	0.4	1.01	126.5	–	–	0.8*	–
12.07.12	0.7	–	>377	251*	629.6***	0.8	–
19.07.12	0.5	–	158.4**	–	–	0.266**	–
26.07.12	0.4	–	>377	–	–	0.125**	0.078
31.07.12	0.6	–	>377	–	–	0.8	–
09.08.12	<LOD	–	>377	–	–	0.8*	–
15.08.12	0.9	–	164.4**	–	–	0.595*	–
24.08.12	1.7*	2.93**	148.6*	–	–	0.8	–
29.08.12	<LOD	–	162.9***	–	–	0.315***	–
05.09.12	<LOD	–	155.8*	–	–	0.121*	–
14.09.12	<LOD	–	129.8**	–	–	0.109*	–
19.09.12	<LOD	–	56.6	–	–	0.154*	–
28.09.12	<LOD	–	<63	–	–	0.12	–

***, p = 0.001, **, p < 0.0001, ***, p > 0.001, [ ], not significant, –, no data available (no sample and/or no measurement, respectively).** Data were compared among themselves and to the data from Skagaströnd 2011 as well as Skagaströnd and Miðfjörður 2012, using one way ANOVA and Duncan's post-hoc test.

2012 Skagaströnd – Temperature (T), Salinity and Chlorophyll *a* (Chl *a*) concentrations in different depths.

Sampling Date	T = 1 m [°C]	T = 3 m [°C]	T = 10 m [°C]	T = 15 m [°C]	Salinity = 1 m [PSU]	Salinity = 3m [PSU]	Salinity = 10 m [PSU]	Salinity = 15 m [PSU]	Secchi depths [m]	Chl a 3 m [µg/g]	Chl a 10 m [µg/g]	Chl a 15 m [µg/g]
26.04.2012	–	–	3.5	–	27.0	–	26.6	–	9.0	–	–	–
04.05.2012	4.3	4.3	3.8***	3.7	22.0	18.7	19.6*	19.0*	13.0	–	–	–
11.05.2012	4.5	4.8	4.6	4.3	10.9*	11.3**	12.5***	12.7***	8.0***	1.27 ± 0.05**	9.33 ± 0.25*	–
18.05.2012	5.0*	4.5	4.1**	4.1	15.6***	15.2*	14.8***	14.6***	12.0	1.04 ± 0.09*	5.52 ± 0.31***	–
23.05.2012	4.9	6.1**	5.0	4.5***	13.9	14.2***	12.8**	12.9**	18.0	0.24 ± 0.02	0.25 ± 0.04	11.5 ± 0.82
31.05.2012	6.1	6.5	5.9	5.5	21.3*	20.8**	21.2***	21.1*	8.0*	0.43 ± 0.02	0.79 ± 0.06	5.59 ± 0.54***
07.06.2012	6.6	5.7***	5.4**	5.3	24.3**	23.9	23.2*	22.9**	8.0	0.31 ± 0.03	0.43 ± 0.02	0.43 ± 0.06*
12.06.2012	5.8	7.0	6.2**	5.8*	29.7	30.7	30.8	30.8	8.0	1.09 ± 0.11**	1.69 ± 0.17**	3.03 ± 0.51***
20.06.2012	7.3	7.8*	7.7	7.6	30.7	30.1	29.4	29.2	8.0	0.77 ± 0.06**	1.27 ± 0.31*	0.73 ± 0.14*
28.06.2012	7.8	8.9	8.6**	8.4	31.6	30.4**	29.3	28.8	10.0	0.52 ± 0.03	1.45 ± 0.10**	2.60 ± 0.28***
04.07.2012	9.0**	11.9	9.9*	8.3***	28.8	28.3	27.2	26.1***	8.0	0.91 ± 0.04	1.02 ± 0.26***	1.98 ± 0.33**
13.07.2012	12.0	12.0**	12.0	10.6	25.0**	24.7*	24.1**	23.8***	7.0	1.50 ± 0.17*	1.34 ± 0.33**	1.94 ± 0.26**
20.07.2012	12.0	12.2	10.4	9.3	26.4**	26.9	26.2	25.3*	5.0	2.83 ± 0.20*	1.44 ± 0.32**	1.33 ± 0.30
27.07.2012	12.6	9.8**	9.5	9.3**	20.5***	20.6***	21.0**	21.2**	8.0**	1.31 ± 0.15**	1.31 ± 0.16**	1.39 ± 0.15*
01.08.2012	9.9**	10.6	10.2	10.1	26.4*	26.2	26.0	25.9	9.0	1.39 ± 0.19	3.08 ± 0.28*	1.19 ± 0.11*
13.08.2012	10.6	12.7	11.6	11.3	25.8	26.0	23.9	23.5	5.0	3.08 ± 0.33*	1.29 ± 0.15	1.74 ± 0.20**
20.08.2012	12.8*	11.7*	10.3***	9.8	28.9	27.9	26.7**	26.3	4.0	2.27 ± 0.14**	1.55 ± 0.10**	1.87 ± 0.24**
31.08.2012	12.0	9.1	9.2	9.1	24.7	24.4	24.9	25.0	4.0	2.78 ± 0.29**	4.26 ± 0.64*	1.18 ± 0.10*
12.09.2012	9.2	8.6	8.6	8.7*	30.6	28.6	26.5	25.9	7.0*	0.61 ± 0.08	1.26 ± 0.11***	1.18 ± 0.08*
25.09.2012	8.6	8.0	8.1	8.2	–	27.1*	27.0	26.7**	5.0	1.52 ± 0.11**	2.69 ± 0.23**	2.55 ± 0.12*
04.10.2012	7.7***	7.6*	7.6***	7.6	–	–	19.6***	19.8**	6.0	0.29 ± 0.01	0.54 ± 0.07	0.65 ± 0.26**
∑	168.7	169.8	162.2	151.5	464.1	456	493.3	461.5	170	24.13	40.5	43.94
Mean	8.44	8.49*	7.7**	7.58*	24.4***	24.0*	23.5**	23.1	8.1**	1.27***	2.13**	2.59*

***, p = 0.001, **, p < 0.0001, ***, p > 0.001, [ ], not significant, –, no data available (no sample and/or no measurement, respectively).** Data were compared to the data from Miðfjörður 2012 as well as Skagaströnd and Miðfjörður 2011, using one-way ANOVA and Duncan's post-hoc test. For Chl *a* concentrations mean values ± SD are included from triplicate measurements.

2012 Skagaströnd – Cell counts at 3 m, 10 m and 15 depth

	Pseudo-nitzschia spp. Cells per L^{-1}			*Dinophysis* spp. Cells per L^{-1}			*Alexandrium* spp. Cells per L^{-1}		
Sampling Date	**3 m**	**10 m**	**15 m**	**3 m**	**10 m**	**15 m**	**3 m**	**10 m**	**15 m**
26.04.2012	300*	460***	880**	0	20	0	0	0	0
04.05.2012	280**	200	320	0	0	0	0	0	0
11.05.2012	40	60	80	0	60	80	0	160	100
18.05.2012	140	500	140	40	40	0	80	80	20
23.05.2012	100	80	0	40	40	40	240*	100**	60***
31.05.2012	440**	440	160	120*	40***	100**	340*	360**	640***
07.06.2012	2340**	900**	440***	0	40	0	100	100*	40
12.06.2012	760***	900**	1600*	560*	140**	20***	880**	700***	660*
20.06.2012	3340*	1620***	2260**	180*	140	180**	180*	120***	280**
28.06.2012	2760*	1100**	740***	620**	180*	160***	40	140**	0
04.07.2012	240**	360*	260***	2040***	1720**	2100*	980**	560***	320*
13.07.2012	140	60	60	1520***	1900*	4420**	3300**	3640*	540***
18.07.2012	460	0	140	1580***	2560*	2240**	120**	420*	80
27.07.2012	800*	640**	40	780*	660***	720**	60**	60*	60
01.08.2012	45280*	0	0	1100**	1160*	1060***	0	0	160*
13.08.2012	45280**	5180*	91880***	1100**	2660*	880***	0	0	0
20.08.2012	3280*	1630**	8540***	340**	420***	760*	0	0	0
31.08.2012	4180*	1710**	840***	140*	120***	240*	0	0	0
12.09.2012	40	60	0	20	20	20	0	0	0
25.09.2012	200**	40	0	0	20	0	0	0	0
04.10.2012	0	0	0	0	0	20	0	0	0
∑	110400	15940	108380	10180	11940	13040	6320	6440	2960
Mean [x 10^2]	52.57*	7.59**	51.61***	4.85	5.69**	6.21*	3.01**	3.07***	1.41*

***, p = 0.001, **, p < 0.0001, ***, p > 0.001, [ ], not significant.** Data were compared to the data from Miðfjörður 2012 as well as Skagaströnd and Miðfjörður 2011, using one-way ANOVA and Duncan's post-hoc test.

2012 Skagaströnd – Toxicity screening – Mussel samples only

	ASP		DSP			PSP	
Sampling Date	**ELISA (BioPol)** DA (ng/g)	**LC-MS/MS (MARLAB)** DA (ng/g)	**PP2A (BioPol)** Total DSP-H (ng/g)	**LC-MS (MARLAB)** Total DSP-H (ng/g)	**LC-MS (MARLAB)** Total DSP-nH (ng/g)	**ELISA (BioPol)** STX (ng/g)	**HPLC (MARLAB)** STX (ng/g)
26.04.2012	0.3	–	–	–	–	0.545**	–
04.05.2012	–	–	–	–	–	0.552**	–
11.05.2012	–	–	<63	–	–	0.555**	–
18.05.2012	2.5***	3.27*	52.8	–	–	0.175	–
23.05.2012	–	–	<63	–	–	0.567**	0.328*
31.05.2012	1.27	–	<63	<LOD	<LOD	0.051	0.287***
07.06.2012	1.43	–	<63	–	–	0.05	–
12.06.2012	4.4**	1.47	<63	–	–	0.05	–
20.06.2012	0.5	–	<63	–	–	0.8**	0.666***
28.06.2012	<LOD	–	114.3**	–	–	0.64	–
04.07.2012	<LOD	–	48.8*	–	–	0.245*	–
13.07.2012	0.3	–	–	–	–	–	–
20.07.2012	0.3	1.68***	165.4***	81.5	152.1**	0.8	–
27.07.2012	1.6***	–	>377	–	–	0.345***	–
01.08.2012	<LOD	–	>377	–	–	0.8	–
13.08.2012	<LOD	–	143.7**	–	–	0.241**	–
20.08.2012	<LOD	–	134.8	–	–	0.154	–
31.08.2012	0.6	–	151.7***	–	–	0.071	–
12.09.2012	<LOD	–	114.7*	–	–	0.8	–
25.09.2012	0.3	–	103.8**	27.9	54.7	0.057	–
04.10.2012	<LOD	–	<63	–	–	0.075	–

***, p = 0.001, **, p < 0.0001, ***, p > 0.001, [ ], not significant, –, no data available (no sample and/or no measurement, respectively).** Data were compared among themselves and to the data from Skagaströnd 2011 as well as Skagaströnd and Miðfjörður 2012, using one way ANOVA and Duncan's post-hoc test.