Proceedings of the 9th Irish Shellfish Safety Scientific Workshop

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INTRODUCTION AND OBJECTIVES OF THE 9th IRISH SHELLFISH SAFETY WORKSHOP

John Evans, Director, Marine Environment & Food Safety Services, Marine Institute

I would like to welcome everyone to the 9th Irish Shellfish Safety Workshop here in Kenmare. The Marine Institute is of course co-sponsored again this year by Bord Iascaigh Mhara and the Food Safety Authority of Ireland. In addition I am pleased to say that this year we have been joined by the Sea Fisheries Protection Authority in the sponsorship of the workshop. My thanks to the sponsors, and also to IFA Aquaculture for securing the venue and publicising along with yesterday's very successful IFA Aquaculture annual conference.

This year we have a very interesting mix of topics including an update on the National Biotoxin monitoring programme, details of a number of research projects with Irish participation and international perspectives on toxin detection. The various presenters today have one thing in common which is finding mechanisms to improve our product and this is as it should be. This may be through improving food safety, increasing productivity or providing easily applied test methods, but in all cases the research being presented is in support of an industry which can and must continuously improve.

I would particularly like to acknowledge the presence today of my predecessor as Director of Marine Environment and Food Safety Services at the Marine Institute, Micheál Ó Cinnéide. Micheál deserves particular credit for his efforts over the past 9 years in supporting and encouraging the use of science to support industry and protect consumer safety. We wish him well in his new role in the Environmental Protection Agency.

Our three sessions today, focusing on a review of the year, research and legislation will be chaired by Joe Silke of the Marine Institute, David Lyons of the Food Safety Authority and Richie Flynn of IFA Aquaculture and I would like to thank them for agreeing to take on this role. Joe also deserves a special word of thanks for organising this year's event.

Finally, I am sure you will all join me in expressing our appreciation to the speakers today for preparing what is a most interesting list of talks. We are grateful for their time and in particular are glad to welcome those that have travelled from abroad. I look forward to hearing what they have to say and hearing the discussion that will follow.

REVIEW OF THE PHYTOPLANTON MONITORING PROGRAMME AND RESEARCH ACTIVITIES IN 2008.

Rafael Salas, Josephine Lyons, Paula Hynes, Tara Chamberlain and Joe Silke

Marine Institute, Phytoplanton Laboratories, Bantry and Galway.

The main items for discussion at this workshop taking aside the annual review of the Phytoplankton monitoring programme, were an update on the recent discovery of the *Azaspiracids* producer organism *Azadinium spinosum* a de-novo producer of this lipophilic toxin compound and an update on the development of molecular tools in the Phytoplankton lab used in the identification of toxin producing algae.

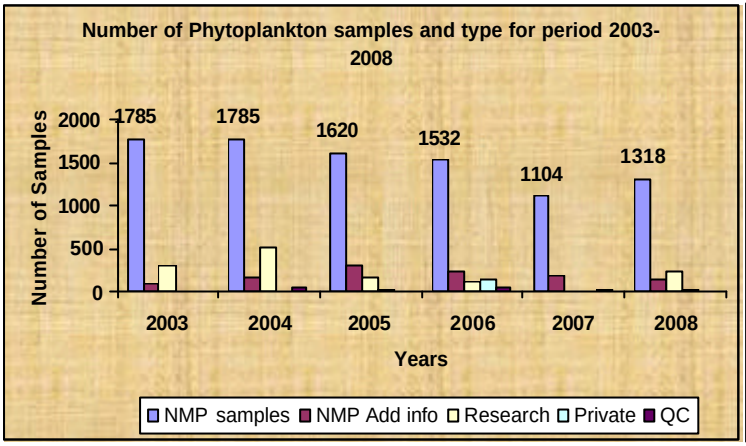
A brief introduction and background of the Phytoplankton National monitoring programme was given to the audience. The programme which is underpinned by the EU directive 854/2004, a Europe wide legislation states that shellfish producing areas have to be monitored for the presence of toxic algae. This monitoring has to be done periodically and the sample needs to be representative of the water column. This last point is very important because it makes reference to the way shellfish areas should be sampled and this point is also a new development from previous legislation in this matter.

The audience was also reminded of the importance of the monitoring programme not only because it services this directive but also because it provides an early warning system on the potential of biotoxin contamination of shellfish going for human consumption, on its cost effectiveness, on the rapid turnaround of results and on the valuable data obtained which may be used to do predictive modelling of bays, of climate change and as an arbitrator in borderline decisions between mouse bioassays and chemistry results.

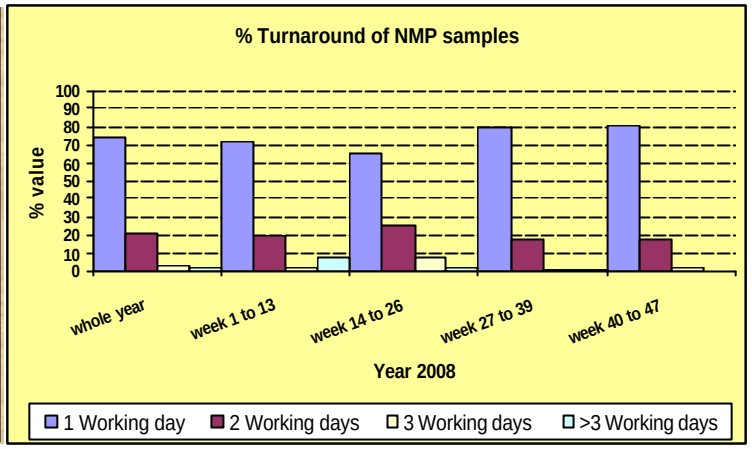
The National Monitoring programme for phytoplankton is a well established programme and this was shown through the improvement and refinement of Phytoplankton shellfish and finfish sites around the country. One important development in the last 2 years has been to increase the number of sentinel sites. A sentinel site is a designated sampling site where a total community Phytoplankton cell count and identification is carried out. The number of sentinel sites has increased from 11 in 2005 to 24 in 2008. This means a better coverage of all the bays around the country.

The number of phytoplankton samples analysed in 2008 has seen an increase from the previous year. In 2007 there was a deep in the number of samples received which was worrying but this trend has stopped in 2008. Graph 1: National Monitoring Programme (NMP) samples 03-08 shows the trend in the last 6 years. What it is obvious from this graph is that the number of samples has decreased over the years and has stabilised around the 1400 samples annually. The reasons for this decrease has been a more focused sampling programme and sample analysis. 85% of the samples are NMP samples. The turnaround of samples is very steady through out the year with 95% of the samples analysed within 2 days of sample receipt (Graph 2) with 70% of the samples analysed within one day of sample receipt.

Graph 1

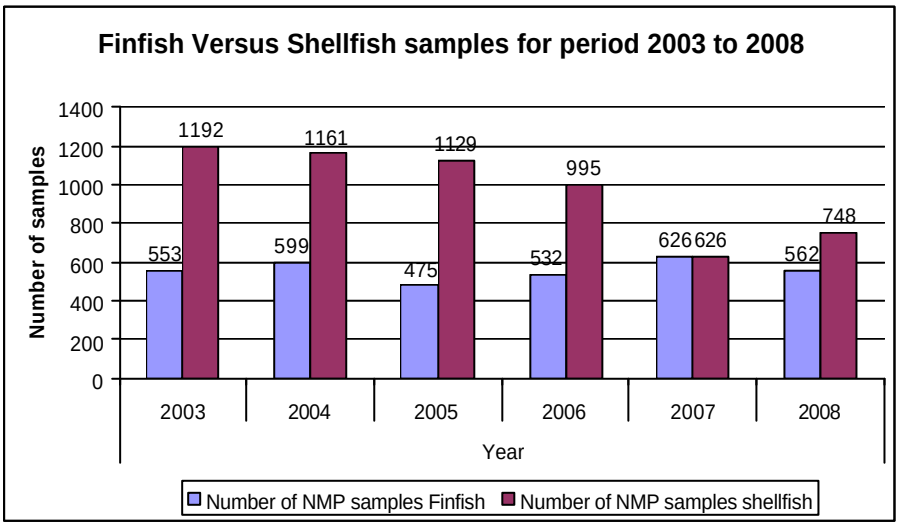


Graph 2



Graph 3 shows an interesting statistic between samples arriving from finfish sites as compared to shellfish sites for the period between 2003 and 2008. This graph shows that while the number of samples for finfish has continued steady over this period, the number of shellfish samples have decreased dramatically. The graph shows a ratio of 2:1 shellfish to finfish samples between 2003 to and including 2006 and this ratio had change to 1:1 shellfish to finfish in 2007. This trend of diminishing shellfish samples had been stopped in 2008.

Graph 3



All these phytoplankton results are quality assured through our accredited Utermohl method for phytoplankton cell counting and identifying, which have gone through the rigorous ISO 17025 Quality Standard. This method is audited annually by INAB, the Irish National Accreditation Board since 2005, when it was awarded to us.

One of the aspects needed to fulfil this accreditation was to participate in a proficiency testing scheme for Phytoplankton. At the time there was no testing scheme similar to quasimeme in the analytical chemistry area on the biological side for phytoplankton. It was through NMBAQC scheme and under Bequalm that the first external phytoplankton intercomparison came to fruition between a number of Phytoplankton monitoring labs in northern Europe, mainly confined to Northern Ireland, Ireland and Great Britain back in 2005.

The Marine Institute Phytoplankton lab has since organised an external intercalibration exercise annually under the umbrella of Bequalm. This exercise had rapidly become the Proficiency testing scheme for phytoplankton enumeration and identification at European level. This is reflected by the increase in participation and by the number of countries already involved in this scheme.

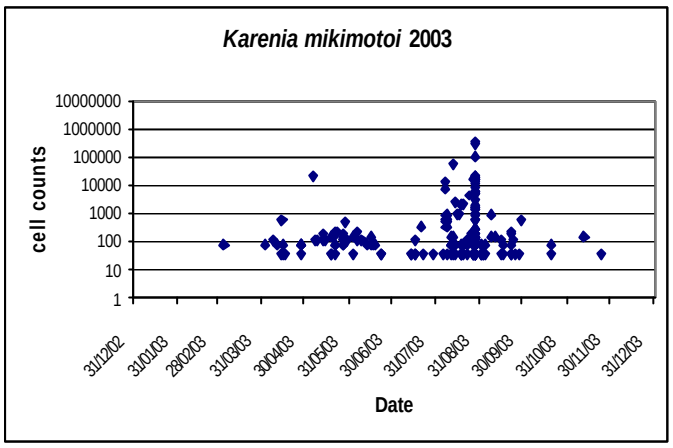
This year for the first time there were 17 labs and 37 analysts across Europe participating in the exercise. Countries like Germany, Holland or Spain are already represented together with Great Britain, Northern Ireland and Ireland.

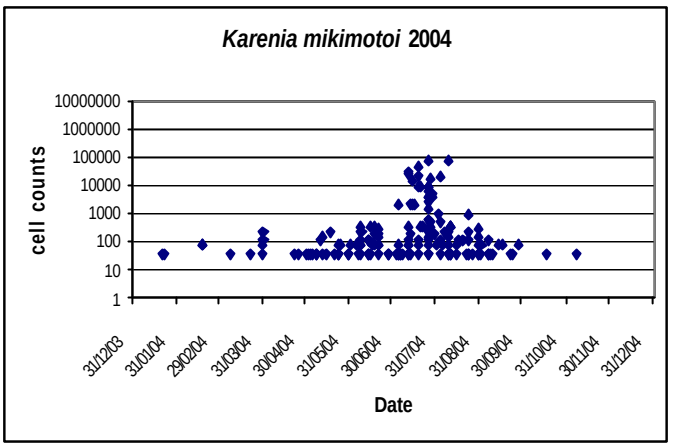
Also, we thought interesting to review the most important HABs species in Irish waters as a way to demystify the belief that HABs only occur in particularly bad years or that are rare events. To prove this theory we embarked on the reviewing of a number of HABs species over a period of 6 years, between 2003 and 2008.
The species that we studied over this period include *Phaeocystis spp. Emiliana huxlei, Karenia mikimotoi* and *noctiluca scintillans.*

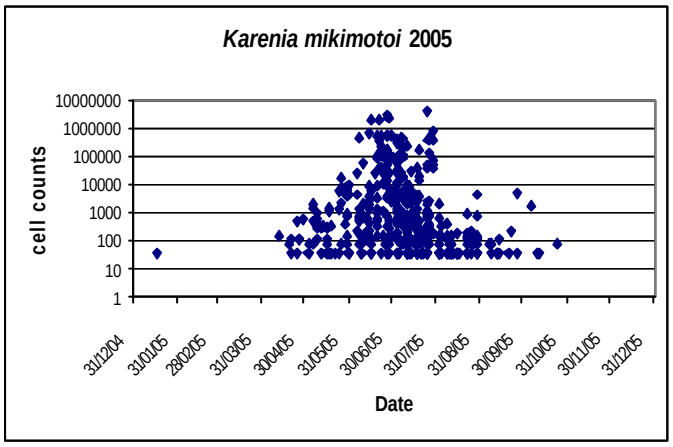
There is clear evidence from Graph 4 using *Karenia mikimotoi* cell counts in logarithmic form as an example that these species are found regularly around the Irish coast year on year that they bloom at some stage and then die down. It is certain that they not always cause harm and that the concentration varies from one year to another, but what is also evident is that they tend to happen in the same time period.
For example *Phaeocystis spp*. Are likely to appear in March-April, while *Noctiluca scintillans* happens at the end of the summer August-September, *Karenia mikimotoi* even though there are found in samples spread through out the year they tend to bloom in mid summer June-July (see graph 4) with this in mind it is easier to predict when these blooms are going to occur, it is not so easy to predict how dense and prevalent they would be and usually other oceanographic processes would have to be looked at in conjunction with this information to build accurate Hab models.

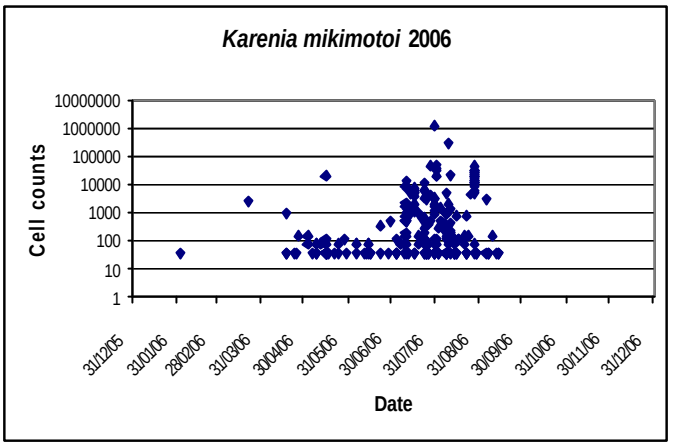
What we know now is that if you find concentrations as high as those found in 2005 for *Karenia mikimotoi* that problems may occur.

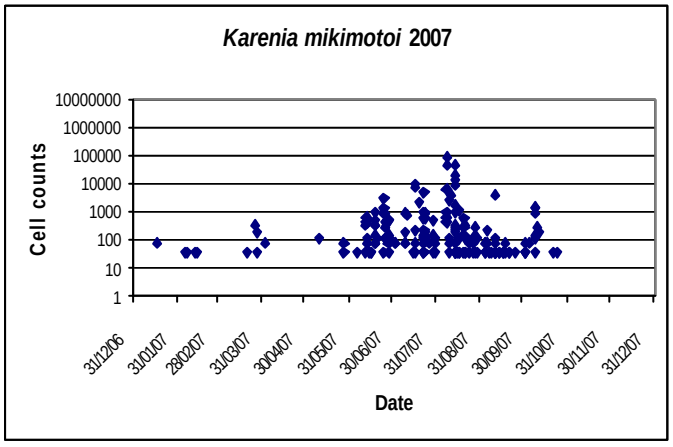
Graph 4: *Karenia mikimotoi* cell counts from 2003 to 2008

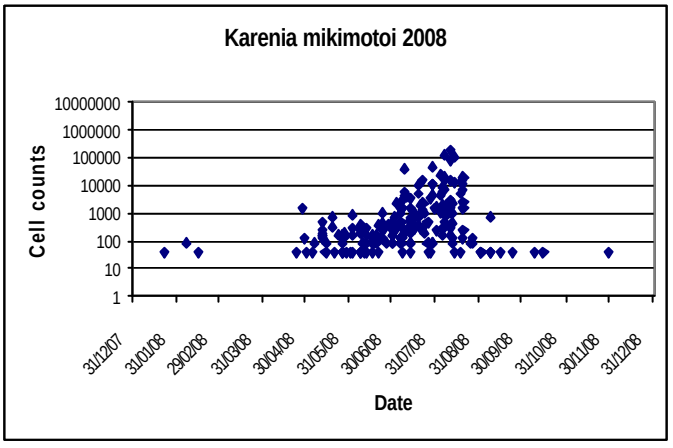












Azaspiracids update

The discovery of the de-novo producer of Azaspiracids, the small thecate dinoflagellate Azadinium spinosum by german scientist Dr. Urban Tillman et al from AWI and given the importance of this toxin in Ireland, this meant that an update on this recent discovery was required.

AZA is a toxin first found because of an intoxication event in Holland back in 1995 where several people got ill from eating contaminated mussels from the west of Ireland.

Originally, thought to be another diarrhetic toxin similar to OA or DTXs, because of having similar symptoms, it was soon realised that this was a novel toxin compound. First associated to Ireland, it was soon discovered to be more widespread than originally thought.

At the time, the thecate dinoflagellate *Protoperidinium crassipes* was thought to be the causative organism of AZA as it was found on the water samples at the time of the event. It is now known, not to be the case. Dr. Urban Tillman et al. during the NORCOHAB survey of the Scottish waters in 2007 and using an LCMS on board were able to detect AZA in plankton size fractions much smaller than that of Protoperidinium.

A new isolate of a small thecate dinoflagellate was finally cultured and shown to be the de novo producer of AZAs. This organism was called Azadinium spinosum, and placed in a new genus of its own, the reason for this being that this organism has taxonomic characteristics of two important group of dinoflagellates, the peridiniales and the goniaulacales but doesn't belong to either. The genus makes a reference to the active part of this toxin the AZA rings and spinosum because of a characteristic antapical spine found in the hypotheca of the cells.

The expectation is now to find and isolate this organism in Irih waters. The NORCOHAB II survey lead by AWI will take place in May this year around the Irish and Celtic Sea and 3 Irish scientists from the Marine Institute will participate.

This survey is part of a wider project ASTOX2 and it is hoped that during the life of this project, the Marine Institute Phytoplankton and biochemistry units will be involved in the culture and isolation of Azadinium spinosum, toxic characterisation and gene probe development.

Molecular tools in the Phytoplankton lab

The Marine Institute Phytoplankton unit has been involved in the past 3 years in partnership with the National diagnostics center (NDC) in NUIG on the development of genetic probes used in the identification of toxic algae.

During these 3 years a number of gene probes for *Pseudonitzschia spp*. and *Dinophysis spp* have been developed for this purpose. Siobhan Kavanagh, Claire Brennan and Majella Maher from the NDC have been paramount to the development of this technique and the technology transfer of this technique to the Marine Institute Phytoplankton unit.

The reasons for using gene probes are various but the most important consideration for the development of such techniques are the difficulty of identifying toxic algae to species level which is crucial for a good Phytoplankton monitoring programme, Gene probes are a good confirmatory method because of its high specificity (melting temperatures). Also, DNA is a very conservative molecule which makes gene probes very reliable tools over time.

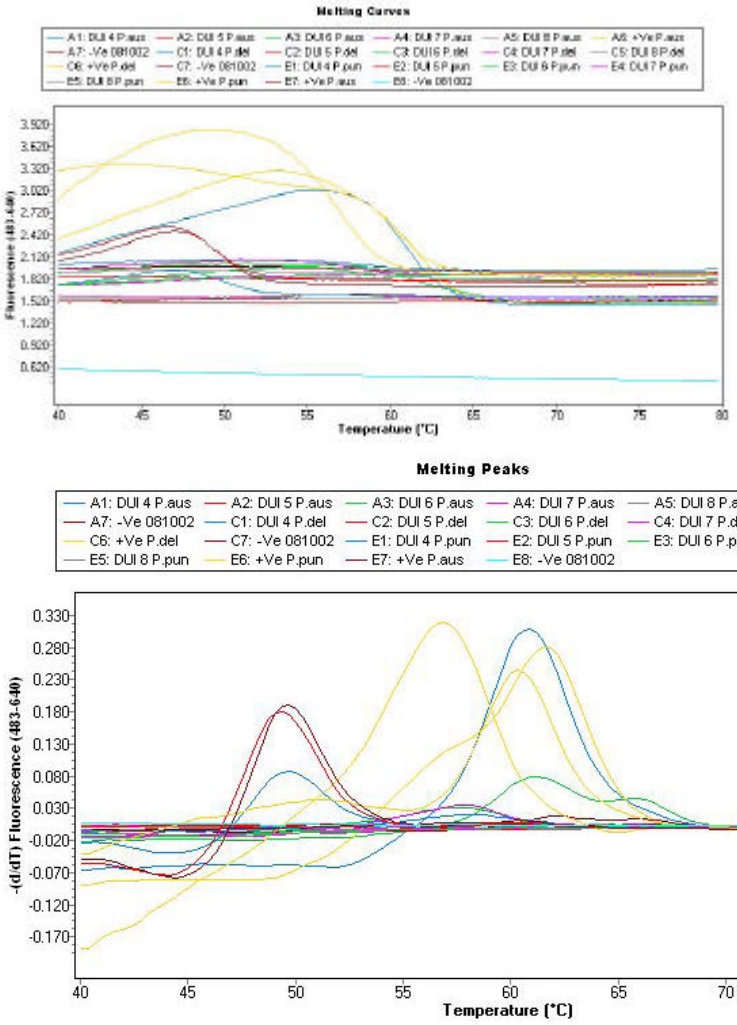
After a series of trials and considerations the preferred method developed is a Real Time PCR assay. This method is currently qualitative but is would be possible to develop further into a quantitative assay if needed.

The advantages of RT PCR over other methods are its sensitivity, specificity and reproducibility, the limit of detection is better than in other assays, the amplification process can be monitored in real-time, and is not influenced by non-specific amplification, the contamination risk is low and the throughput of samples is fast. So a good number of samples can be processed in a very short time period.

The next steps are to validate this methodology and possibly accredit the method through INAB. Work in the development of more gene probes for *Pseudonitzschia spp* , *Azadinium spinosum* and *Alexandrium spp.*

This method could become a confirmatory method for the Phytoplankton programme, which will work as a risk management tool and early warning system for the biotoxins programme.

Graph 5 & 6: Melting curves and melting peaks of *Pseudonitzschia spp.* probes in NMP phytoplankton samples



A Review of Shellfish Toxicity Monitoring in Ireland for 2008

Dave Clarke[1], Conor Duffy[1], Joe Silke[1], Leon Devilly[1].

Laboratory staff from MI Bioassay and Biotoxin Chemistry Units[1]

Laboratory staff from En-Force Laboratories Ltd.[2] & Charles River Biological Laboratories Europe[3].

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[2] En-Force Laboratories Ltd, Unit G Ballyvolane Ind Est., Spring Lane Cork.

[3]Charles River Biological Laboratories Europe, Foxford Road, Ballina, Co. Mayo.

The National Marine Biotoxin Monitoring Programme for shellfish is co-ordinated by the Marine Institute's National Marine Biotoxin Reference Laboratories based in Galway, under Service level agreements with SFPA and FSAI.

Samples of shellfish species are routinely analysed for the presence of marine biotoxins by biological and chemical methods in accordance with Commission Regulation (EC) No. 1664/2006, Regulation (EC) No. 853/2004 and Regulation (EC) No. 2074/2005.

The Marine Institute (MI) as National Reference Laboratory (NRL) for Marine Biotoxins are required as part of their NRL duties under Council Decision 93/383/EEC, *of 14 June 2003 on reference laboratories for the monitoring of marine biotoxins*, to coordinate the activities of the National Laboratories in respect of Biotoxin analysis under the National Biotoxin Monitoring Programme which includes the organisation of intercomparison exercises and the regular auditing of the National Laboratories, En-Force Laboratories and Charles River Biological Laboratories.

The MI also participates in a number of proficiency testing schemes and intercomparison exercises including Quasimeme, BEQUALM, with the Community Reference Laboratory and also a number of individual laboratories, organisations and institutes. All test methods for the analysis of shellfish toxins via bioassay and chemical analysis remain accredited to ISO 17025 standards during 2008.

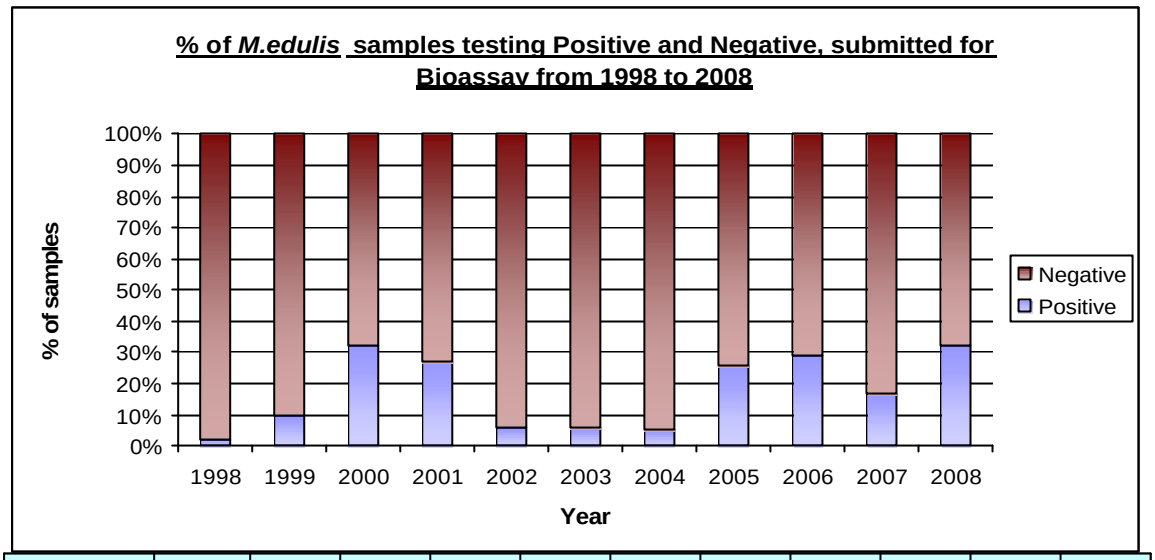
Overview of Biotoxin Events in 2008

Table 1. illustrates the numbers of samples / tissues analysed via biological and chemical methods for Azaspiracid Shellfish Poisons (AZP), Diarrhetic Shellfish Poisons (DSP), Amnesic Shellfish Poisons (ASP) and Paralytic Shellfish Poisons (PSP) over a 7 year period from 2002 – 2008.

	PSP Bioassay Immunoassay	**DSP** Bioassay	**DSP & AZP** via LC-MS/MS	**ASP** (Scallops) via HPLC	**ASP** (other species) via HPLC
2002	**114**	**2854**	**2844**	**656**	**33**
2003	**120**	**2684**	**2709**	**658**	**56**
2004	**145**	**2252**	**2252**	**669**	**92**
2005	**243**	**2549**	**2549**	**469**	**379**
2006	**148**	**2404**	**2387**	**539**	**180**
2007	**165**	**1898**	**2151**	**506**	**125**
2008	**139**	**1838**	**1923**	**506**	**133**

Table 1. Number of analyses via different methodologies 02 - 08

Overall for 2008, 32% of all *M.edulis* samples submitted and analysed (illustrated in Table 2), were found to be positive via Bioassay (n = 1117) compared to 17% for 2007.



	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
% Positive	2.5%	11.2%	32.7%	26.7%	6.03%	5.89%	5.06%	25.8%	29.3%	16.5%	32.3%
Highest OA µg/g	NA	NA	NA	NA	3.81	0.57	3.83	4.00	0.29	0.07	0.92
Highest AZA µg/g	NA	NA	NA	NA	0.23	0.19	0.02	>ULQ	1.63	1.4	>ULQ

Table 2. % of M.edulis samples +ve & -ve and highest chemical concentrations observed 1998 - 2008

Tables 3 & 4 illustrate the number and locations of closures observed due to DSP and AZA toxins for 2008 and 2007. For 2008 there was 23 site closures predominantly in the SouthWest, and also in the West and North West, compared to 20 site closures in 2007.

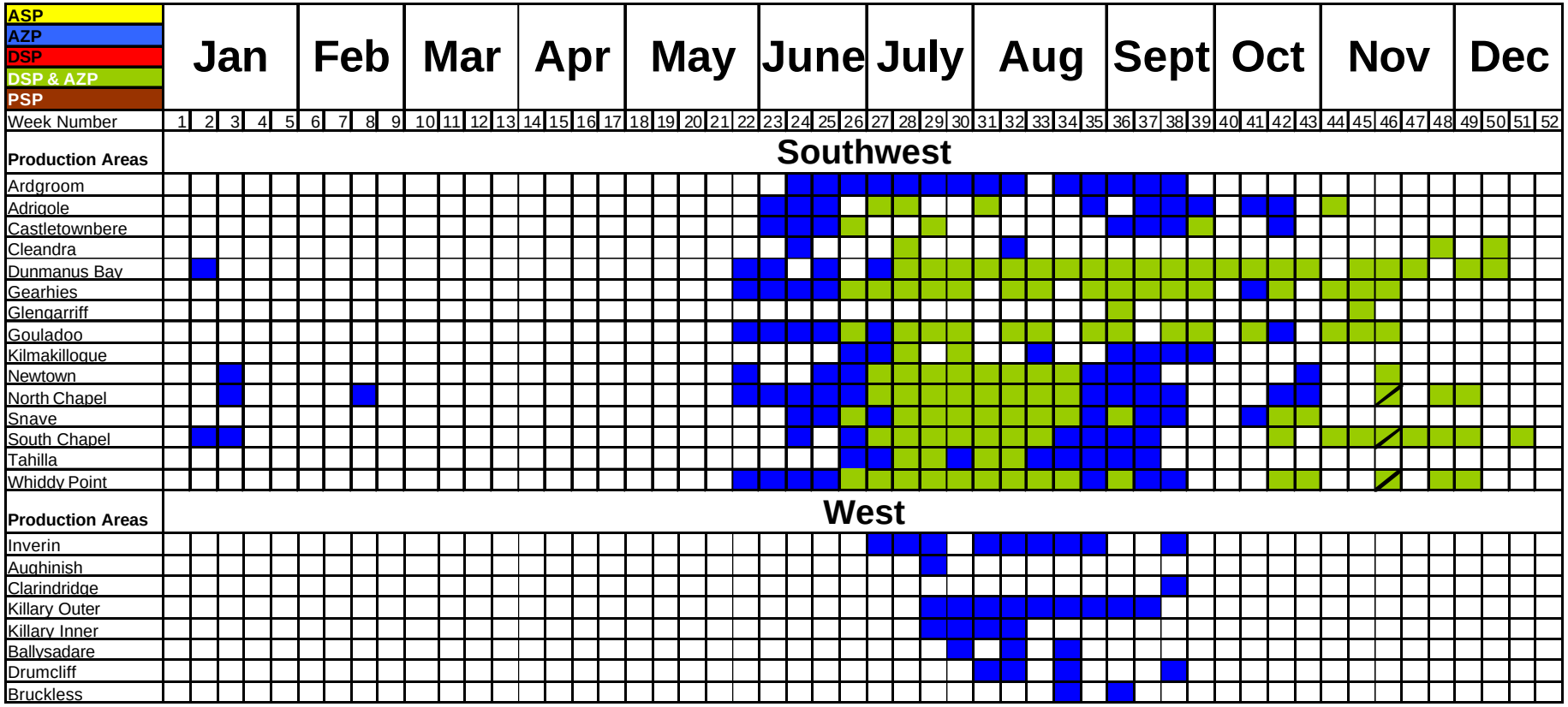


Table 3. Site Closures for 2008 via site and causative toxin group

23 Site Closures in 2008

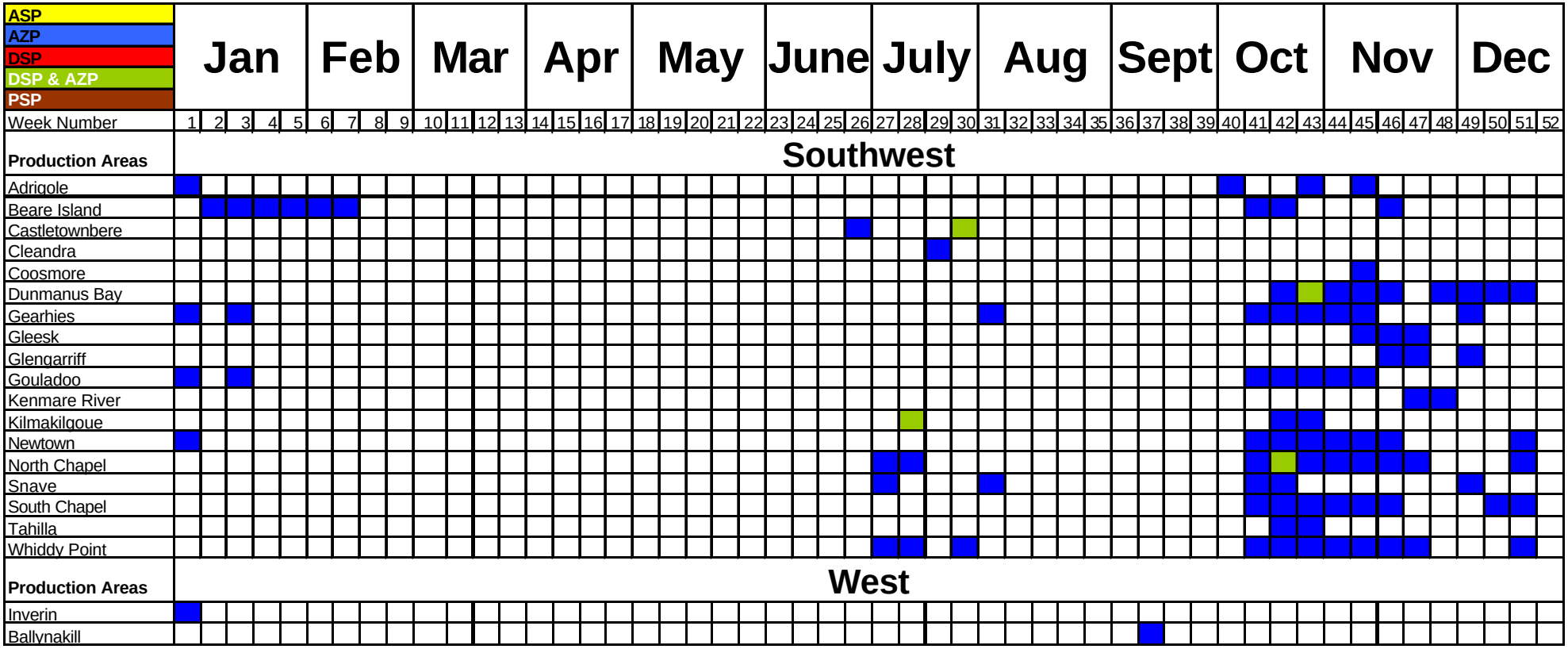


Table 4. Site Closures for 2007 via site and causative toxin group

20 Site Closures in 2007

Azaspiracid (AZA) Toxicity

During 2008 AZA toxicity persisted in samples of *M.edulis* for a small period throughout January, and resulted in closures in a number of sites within Bantry & Dunmanus above the regulatory level of 0.16µg/g^{-1} Total Tissue, as a carry over of the AZA event which began in Oct 2007. AZA concentrations were observed to decrease further to levels below the regulatory level during January resulting in Open status being assigned.

From the end of May 2007, AZA levels were observed to increase in samples of *M.edulis* from sites within Bantry and Dunmanus, and from June in sites within Kenmare to concentrations above the regulatory limit. The highest AZA concentrations above the Upper Limit of Quantification (>ULQ = > 1 µg/g^{-1} Total Tissue) were observed in these sites during July – September. ZA concentrations were also observed above the regulatory level in samples of *M.edulis* from Galway Bay, Killary Harbour, Drumcliff and Ballysadare during July and August, and also in Bruckless in August and September.

From October onwards, AZA concentrations were observed to decrease nationally, resulting in previously affected sites in the West and NorthWest being assigned Open status. In the affected sites in the SouthWest, concentrations were observed to decrease to levels < ULQ, however remained above the regulatory level. Further decreases were observed in November to below the regulatory limit, where the majority of sites within Kenmare were assigned Open status, and during December the remaining affected sites within Bantry and Dunmanus were assigned Open status.

National AZA concentration levels are illustrated in *Fig. 1*, and represented geographically in *Fig. 2.*

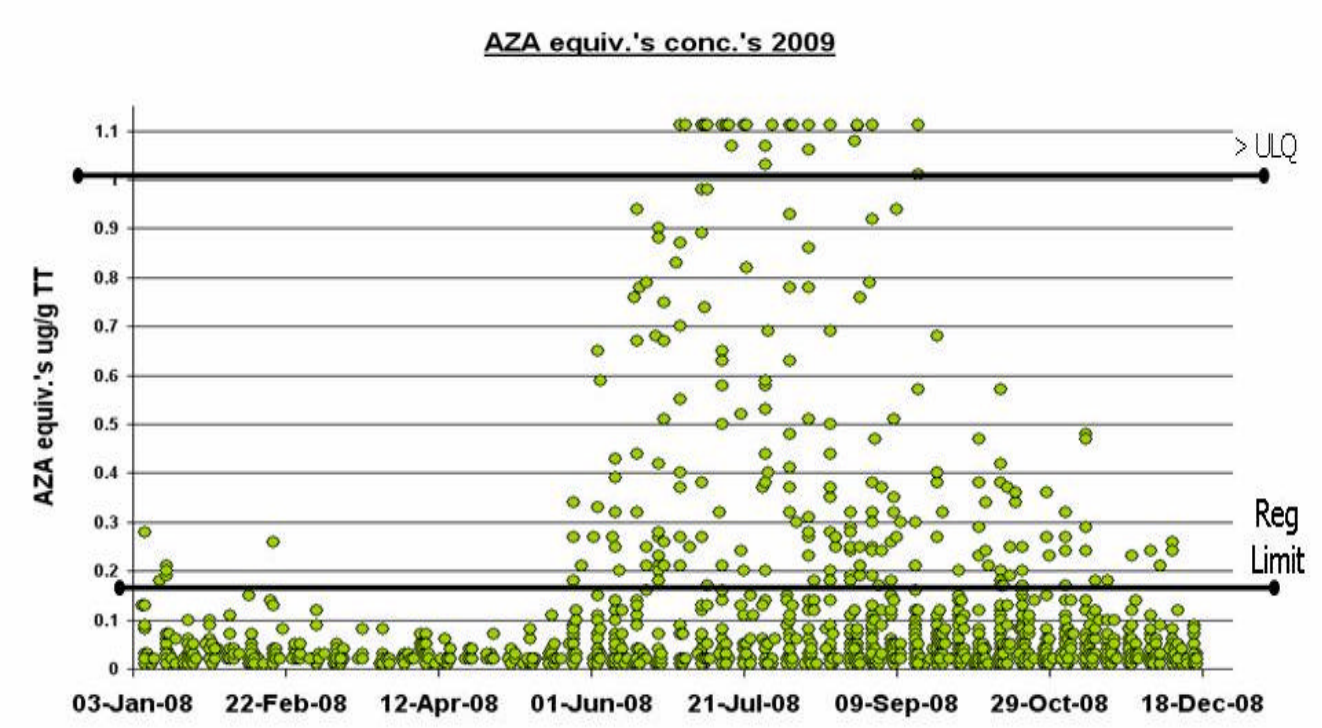
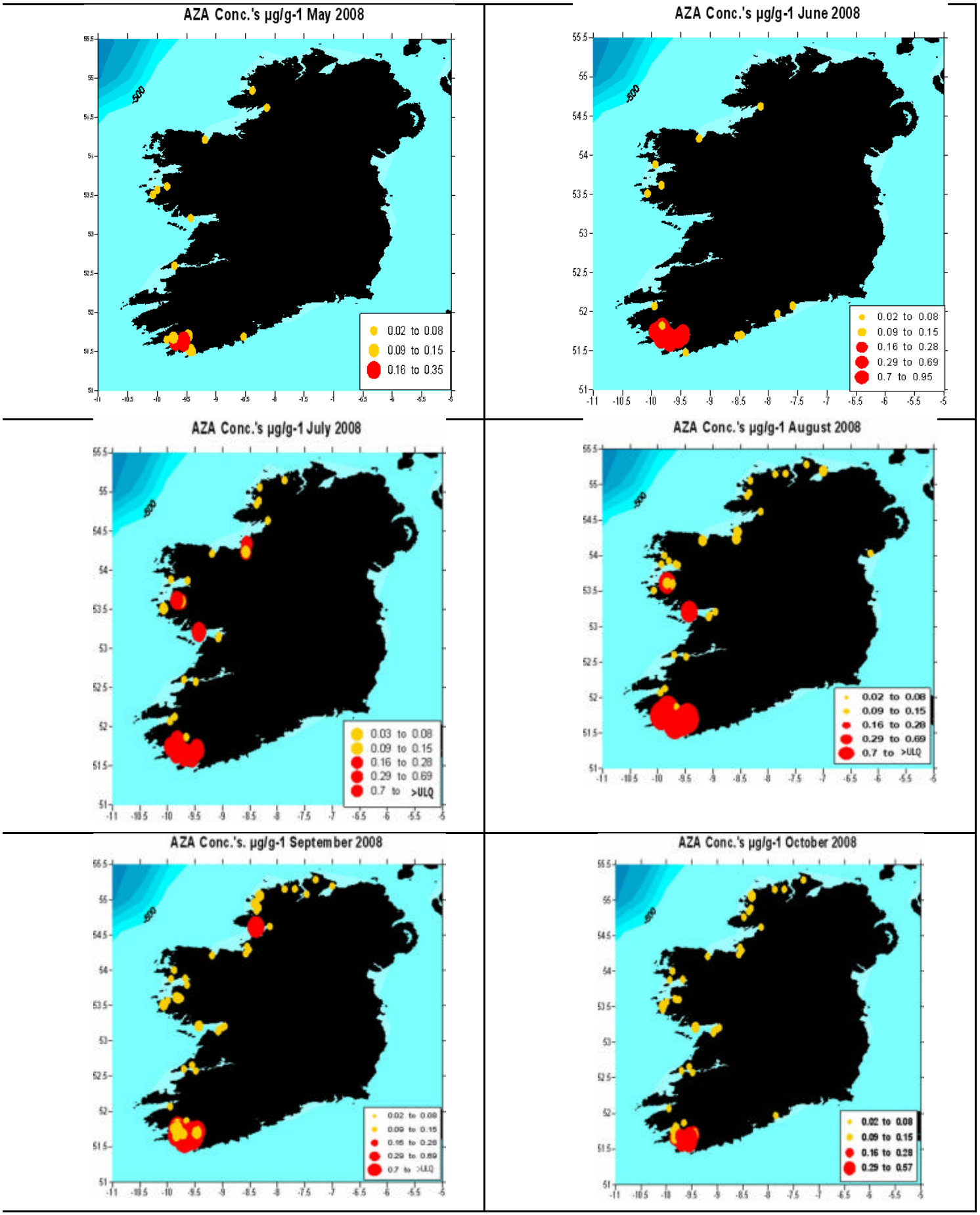


***Fig. 1*. Total AZA's concentrations (AZA equiv.'s) in mg/g TT^{-1} Nationally**

Fig. 2. AZA Results May – Oct 08 Total Azaspiracids AZA's 1, 2 & 3 in µg/g TT^{-1}



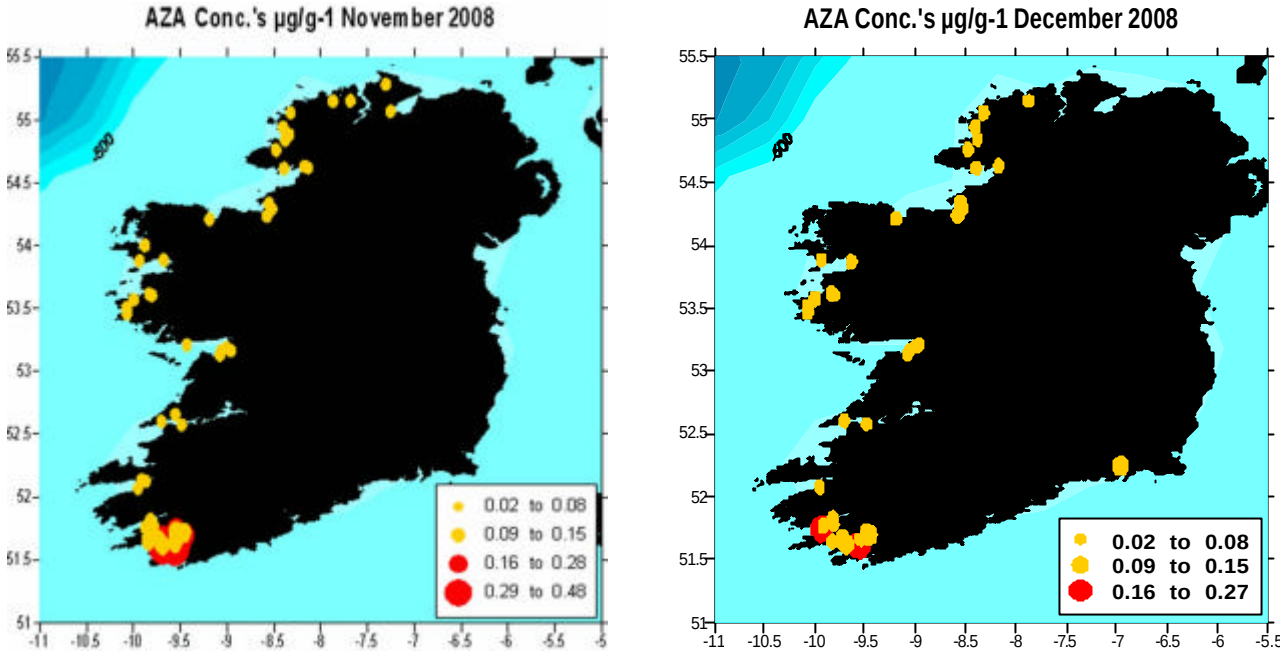


Fig. 2. cont. **AZA Results Nov – Dec 08 Total Azaspiracids AZA's 1, 2 & 3 in mg/g TT^{-1}**

Diarrhetic Shellfish Toxicity (DSP)

From Mid July, DSP concentrations were observed to increase above the regulatory level in samples of *M.edulis* from Bantry and Dunmanus. These concentrations increased during August in these, where the highest concentration of 0.92 µg/g^{-1} Total Tissue was observed. DSP concentrations decreased during September, though concentrations did not significantly increase or decrease during October and November. Further decreases in concentrations were observed in December enabling the previously affected sites to be assigned Open status. The predominant quantifiable DSP toxin in samples from July to August was Okadaic Acid (OA), and from late August onwards the predominant DSP toxin present was *Dinophysis* Toxin 2 (DTX-2).

National DSP concentration levels are illustrated in *Fig. 3*, and represented geographically in *Fig. 4.*

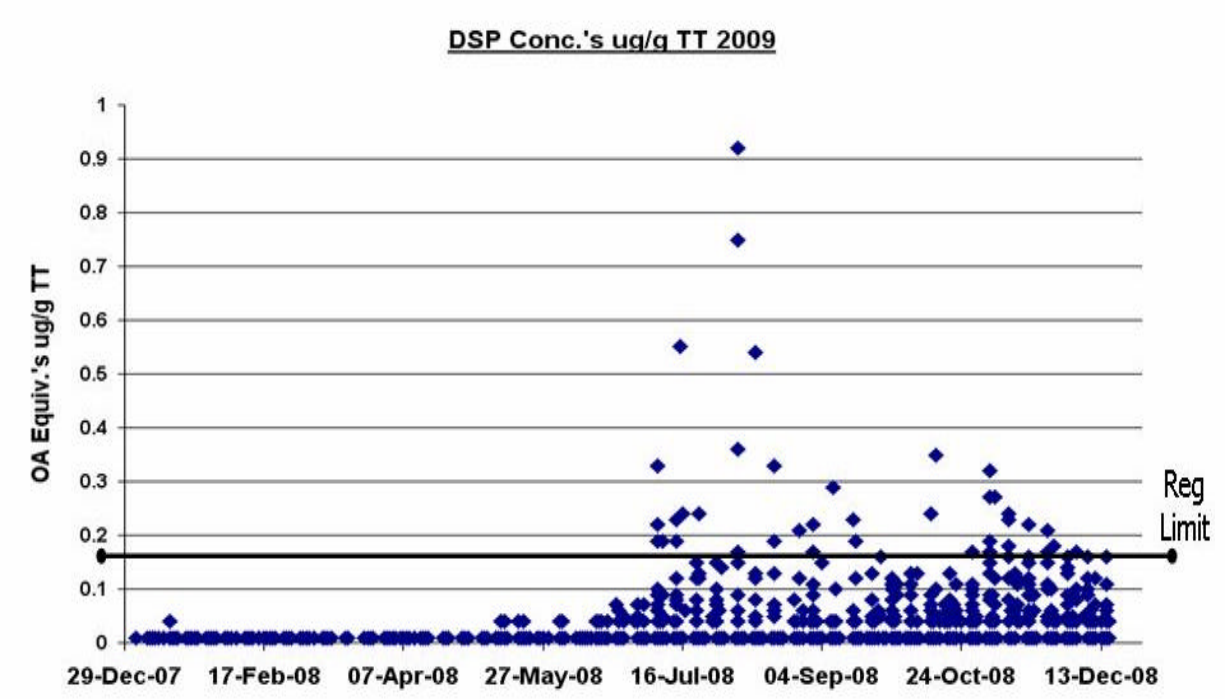
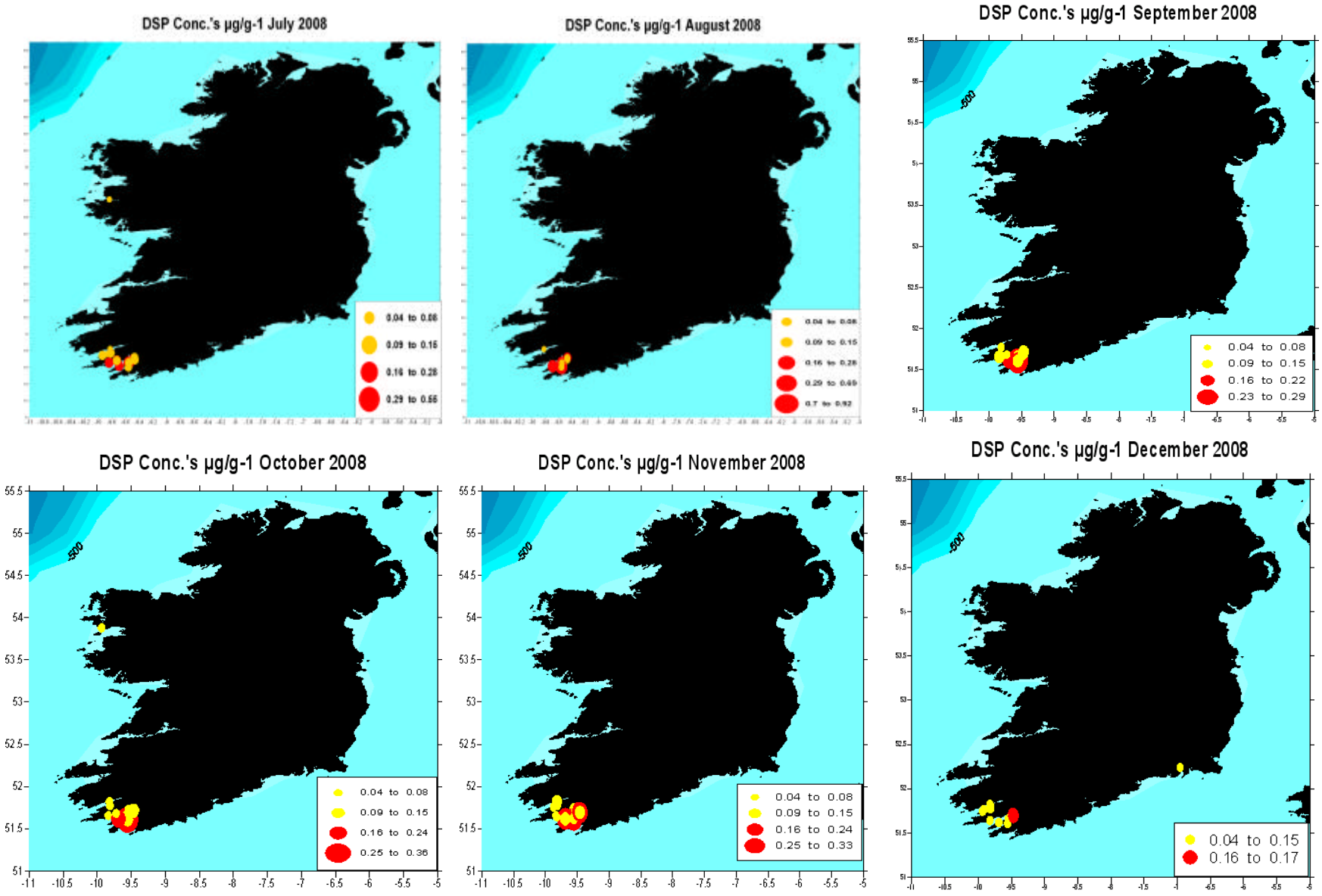


Fig. 3. **Total DSP concentrations (OA Equiv.'s) in mg/g TT^{-1} Nationally**

Fig. 4. **DSP Results May – Oct 08 Total Okadaic Acid,** ***Dinophysistoxins*** **1 & 2 in mg/g TT^{-1}**



Amnesic Shellfish Poisoning

During 2008, **506** analyses for ASP were conducted on Scallop tissues (*P. maximus*), typically Gonad and adductor muscle tissues, where the levels observed on Adductor Muscle tissues (240 analyses) were all below the regulatory limit (highest level observed **7.7 µg/g^{-1}**).
1 of **238** Gonad tissues analysed were observed to be above the regulatory limit **> 20 µg/g^{-1}**, where the highest level observed was **29.6**µg/g^{-1}.

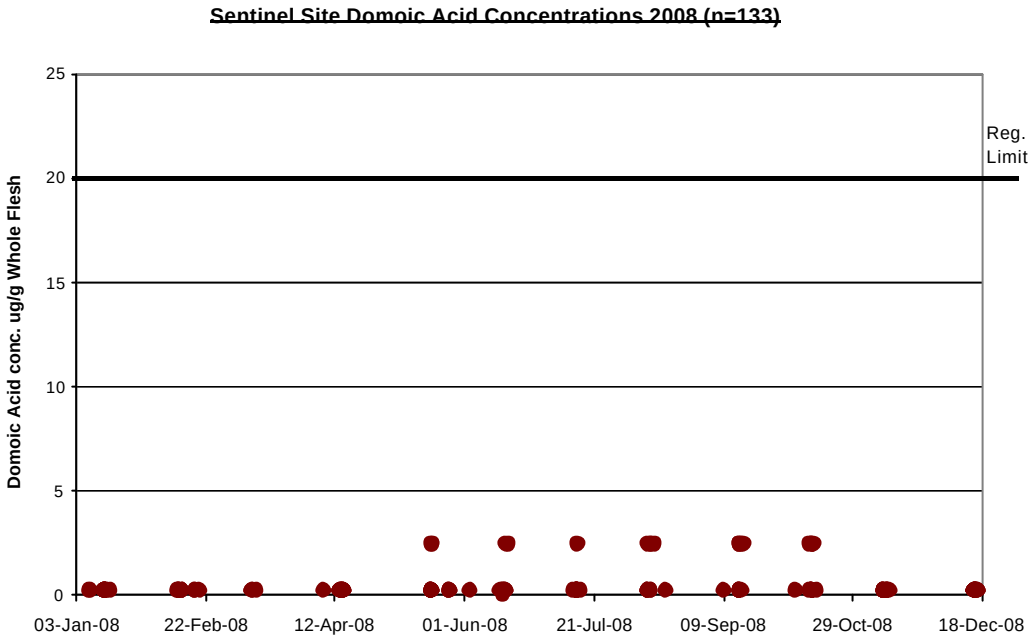


Fig. 5. Domoic Acid conc.'s in Shellfish samples (except scallops) for 2008

Additionally during this time period, a total of 133 samples of *M.edulis, C.gigas, O.edulis, E.siliqua & T.philippinarium,* were analysed for the presence of Domoic & Epi-Domoic Acid (DA). All samples analysed were observed to be either **< Limit of Detection (<LOD) or < Limit of Quantification (LOQ)** ***(Fig. 5.)***.

Paralytic Shellfish Poisoning (PSP)

During 2008, **139** samples of *M.edulis, C.gigas, O.edulis, E.siliqua & T.philippinarium*, were submitted for PSP analysis. All samples were **<LOQ** via AOAC PSP Bioassay.

Review of the Shellfish Microbiology Programme 2008

Bill Doré[1] and Brian Nolan[2]

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Introduction

Bivalve molluscan shellfish can accumulate micro-organisms when grown in sewage contaminated water. When such shellfish are eaten raw or lightly cooked they can present a public health risk for consumers. Regulatory controls exist to limit these risks and an extensive microbiological monitoring programme exists in Ireland to support these controls. Statutory Instrument 335 of 2006 transposes EU Regulations on shellfish safety into Irish law. Under these regulations the quality of shellfish harvest areas are required to be determined and all commercial bivalve shellfisheries in Ireland are monitored for levels of *Escherichia coli.* On the basis of this monitoring each area is classified into one of three categories which determine the level of treatment that is required before consumption. These categories and associated acceptable treatment are set out in table 1.

Table 1. Shellfish Classification based on E. coli monitoring

Category	Microbiological Standard (MPN 100g^{-1} shellfish flesh)	Treatment required
Class A	<230 *E.coli*	May go direct for human consumption
Class B	<4,600 *E.coli* (90% compliance)	Must be depurated, heat treated or relayed to meet class A requirements
Class C	<46,000 *E.coli*	***Must be relayed for 2 months to meet class A or B requirements or may also be heat treated***

The Sea Fisheries Protection Authority (SFPA) is the Competent Authority for shellfish hygiene controls in Ireland and the Marine Institute (MI) is the National Reference Laboratory (NRL) for microbiological monitoring of shellfish. The SFPA and MI work jointly to deliver the programme for monitoring shellfish in Ireland. Procedures for monitoring and undertaking classification of shellfish harvesting areas are described in a recently developed code of practice which is available from the SFPA website http://www.sfpa.ie/. This paper highlights the procedures and respective roles of both these bodies involved in the programme. The monitoring programme in 2008 is reviewed and technical progress made by the NRL in 2008 is briefly highlighted.

Microbiological monitoring

Seafishery Protection Officers are responsible for collecting samples for shellfish for classification purposes. The number of sampling in each Shellfish harvesting areas varies depending on the size of the area and number of species harvested. In general a sample is taken for *E. coli* analysis every month. In 2008 1734 samples were taken for analysis for *E. coli* representing an increase of around 10% on the number of samples taken in 2007. It is of critical importance that the results used to make the decisions during the classification process can be relied upon to be correct and considerable effort is put into quality control of both the sampling and testing.

All samples collected by Seafisheries Protection Officers must conform to standard criteria if they are to be accepted for analysis. All samples must be temperature controlled and maintained below 15ºC during transit to the laboratory. Samples must be received in the laboratory within 24 hours of sampling. Alternatively samples transported to the laboratory within 4 hours do not have to be maintained blow 15ºC. In 2008 only one sample was rejected because these conditions were not met.

Quality control of analysis

To control the quality of sample analysis only laboratories which are accredited to ISO 17025 by the Irish National Accreditation Board (INAB) for the analysis of *E. coli* using an ISO method (ISO TS 16449) are used to test shellfish samples. In 2008 5 laboratories were contracted by the MI to undertake *E. coli* analysis for the classification programme. The MI also carried out analysis for the programme. The laboratories are distributed around the country to ensure delivery of samples is possible within the prescribed time period. As the NRL the MI responsible for ensuring the quality of results used for the classification of harvesting area.

To ensure the quality control of analysis, each laboratory is required to participate in the UK Health Protection Agency organised External Quality Assurance (EQA) scheme. In 2008 three distributions of bacterial cultures were analysed by each laboratory as part of this scheme. In addition the EU Community Reference Laboratory for microbiological monitoring in shellfish organised a whole animal ring trial using naturally contaminated category B mussels. The results for the Irish laboratories participating in these schemes are presented in Table 2.

Table 2. Results of quality assurance schemes by national Laboratories for *E. coli* analysis in shellfish for classification purposes

Lab.	HPA EQA Scheme (*E. coli* cultures) (% score)			CRL Whole animal (MPN 100g-1)	
	March	**July**	**Nov**	**Replicate 1**	**Replicate 2**
1	100	100	100	1700	2400
2	**87.5***	100	100	310	750
3	100	100	100	310	500
4	100	100	100	3500	1100
5	100	100	100	500	500
6	100	100		1300	1100

*Result >3SD from median value

As part of the HPA EQA scheme, just one laboratory scored <100% on one occasion. This result represented value that was greater than 3 times the standard deviation of the median MPN result obtained by all participants. This remains an acceptable result and within the statistical variation possible within the MPN test. Such a score serves merely as flag and would indicate poor performance if there was a continuing trend. For the whole animal ring trial all laboratories performed well. All results from both replicates in this ring trial gave the expected category B result.

Classifications in 2008

In 2008 the initial annual classification review was conducted by the SFPA and agreed by the microbiological sub committee of the molluscan shellfish safety committee in June. This was first year that classifications were determined using the new code of practice on microbiological monitoring. The most significant difference during this year's process was that the classifications were determined based on the previous three years data for each harvesting areas. Previously classifications were determined every six months based on the previous year's data. The aim of using three years data is to accurate assessment of the background contamination in the harvesting which is the overall aim of the classification process. Even though 2008 had a 10% increase on the number of Microbiobgical samples taken in 2007, the 2008 microbiological monitoring programme saw a significant, 25%, reduction in the number of Elevated/Out of range results recorded for production areas. A further development is that the code of practice allows for seasonal classifications to be given in areas where the data shows a clear seasonal trend in E. coli levels over the three year period. Twelve such seasonal classifications were given in 2008.

Classified Bivalve Mollusc Production Areas in Ireland (6th of April 2009)

Classified Bivalve Mollusc Production Areas in Ireland: 6th of April 2009

I	II	III	IV	V	VI
Production Area	**Boundaries**	**Bed Name**	**Species**	**Classification**	**Notes**
Lough Foyle	Magilligan Head to Inishown Head	All Beds	Mussels Oysters	B B	
Tra Breaga	Malin Head to Dunaff Head	All Beds	Oysters	B	
Lough Swilly	Fanad Head to Dunaff Head	All Beds	Mussels Oysters	B B	
Mulroy Bay	Melmore Head to Ballyhoorisky Point	All Beds	Mussels Oysters	A A	
Sheephaven	Rinnfaghla Point to Horn Head	All Beds	Mussels	A	Seasonal classification 1 Jan to 30 April reverts to class B at other times (Note 1).
			Oysters	B	
Gweedore	Carrick Point to Carrickacuskeame and Torglass Island to Dunmore Point	All Beds	Oysters	B	
			Cockles	B	Preliminary classification (Note 2)
Dungloe	Wyon Point to Burtonport Pier	Dungloe	Oysters	B	
Traweenagh	Dooey Point to Crohy Point	All Beds	Oysters	A	
Gweebarra	Gweebarra Point to Cashelgolan Point	All Beds	Oysters	A	
Loughras Beg	Loughras Point to Gull Island	All Beds	Oysters	B	

McSwynes Bay	Carntullagh Head to Pound Point	Bruckless	Mussels	A	Seasonal classification 1 Dec to 30 Jun reverts to class B at other times (Note 1).
Inver Bay	St. John's Point to Doorin Point	All Beds	Mussels	A	
Donegal Harbour	Doorin Point to Rossnowlagh Point.	All Beds	Oysters Mussels	B B	
Drumcliff Bay	Raghly Point to Deadman's Point	All Beds	Mussels Oysters Clams Cockles	B B B B	
Sligo Harbour	Deadman's Point to Killaspug Point	All Beds	Oysters Clams	B B	
Ballysodare Bay	Killaspug Point to Derkmore Point	All Beds	Mussels	B	
Killala Bay	Ross Point to Iniscrone Point	All Beds All licensed Beds	Mussels Oysters	B B	
Blacksod Bay (Belmullet)	Blacksod Point to Kanfinalta Point	All Beds	Oysters	A	
Achill North	Kinrovar Point to Ridge Point and Achill Bridge East to Achill Bridge West	All Beds	Oysters	A	Seasonal classification 1 Dec to 31 Mar, reverts to class B at other times (Note 1).
			Mussels	B	
Achill South	Achill Bridge east to Achill Bridge West and Bolinglanna to the Southernmost Point of Achill Beg	All Beds	Oysters	A	Seasonal classification 1 Oct to 30 Apr, reverts to class B at other times (Note 1).

Clew Bay	Area within a one nautical mile (1,852 M) radius of Roskeen Point (53° 53.46'N, 09° 40.10' W)	Tieranaur Bay	Oysters	A	Seasonal classification 1 Jan to 30 Jun, reverts to class B at other times (Note 1).
	Area bounded to the west by a line from Mulranny Pier to Old Head and to the south east by 09° 35.37' W and to the north east by a line due north and east respectively from the point at which 09° 37' W and 53° 52.60 N intersect	Corrie Channel and Rosslaher Beds	Mussels Oysters	B B	
		All other Beds	Mussels Oysters	A A	
Killary Harbour	Rusheen Point to Rossroe Quay	All Beds	Mussels	B	
Ballinakill	Renvyle Point to Cleggan Point	All Beds	Mussels Oysters	B A	
Streamstown Bay	Gubarusheen Point to Omey House ruins to Ardoe	All Beds	Oysters	A	
Clifden Bay Inner	Errislanan Pier to Dooghbeg Quay (ruins)	All Beds	Mussels	B	
Clifden Bay outer	Errislanan Pt to western most point of Turbot Island to westernmost point of Ardmore Island and from Errislanan point to Dooghbeg Quay (ruins)	All Beds	Clams	B	Preliminary classification (note 2)
Mannin Bay	Errislanan Point	All Beds	Oysters	A	

	to Knock Point.				
Kilkerian Bay North	Area bounded to the North, Kylesalia Creek to Northern edge Crow Island: To the East, Garrivinnagh Quay to Leighon Island; and to the South, Kilkieran Point to the Southern Edge of Inchagaun Island.	Kilkieran North	Oysters	A	Preliminary Classification (Note 2)
Casheen Bay	Dinnish Point North to Green Island off Illauneeragh	Casheen Bay	Mussels	A	Preliminary classification (note 2)
Galway Bay	Cloghmore Point to a point at 53°11' 00" N, 9° 30' 00" W to a point at 53°11' 00" N, 9° 24' 00" W. to Loughaunbeg Point.	Inverin	Mussels	B	Preliminary classification (Note 2)
	Ardfry Point to Kilcolgan Point	Mweeloon Bay	Mussels Oysters	B A	
	Kilcolgan Point to Deer Island to Aughinis Point Excl Kinvarra Bay.	Corraduff Beds Clarenbridge and Killeenaran Beds	Oysters Mussels Oysters	A B A	
		Clarenbridge	Clams	B	Preliminary classification (Note 2)
	Knockapreaghaun Point to Goragh Island to Traught Point (8° 59.1' W and 53° 10.4' N.)	Kinvarra Bay	Mussels Oysters	B B	

	Aughinis Point to New Quay	Aughinis	Oysters	A	Seasonal classification 1 Oct to 31 Mar reverts to class B at other times (Note 1).
	Finnivarra Point to Muckinis Point	Poulnaclough Bay	Mussels	A	Seasonal classification 1 May to 31 Aug reverts to class B at other times (Note 1).
			Oysters	B	
Carrigaholt	Kiloher Head to Leck Point and Corlis Point to Beal Point	Carraigaholt	Oysters	A	Seasonal classification 1 Dec to 31 Mar, reverts to class B at other times (Note 1).
		Rinneville Beds& College Bay Beds	Oysters	A	
Poulnasharry	Corlis Point to Baurnahard Point	All Beds	Oysters	A	
Askeaton	Area bounded by a line from Knockfinglas to Beal Point to Corlis Point to Baurnahard Point to Crusheen Point to Aylevaroo Point	All Beds	Oysters	B	Preliminary classification (Note 2)
Ballylongford	Beal Point to Knockfinglas Point	All Beds	Oysters	A	
Tralee Bay	Kerry Head to Brandon Head	All Beds	Oysters	B	
Castlemaine Harbour	Inch Point to Rossbeigh Point	All Beds	Mussels	B	
			Oysters	B	Preliminary classification

					(Note 2)
			Clams	B	Preliminary classification (Note 2)
Valentia River	Bray Head to Reencaheragh Point and Douglas Head to Fort Point	All Beds	Oysters	B	
Kenmare River	Lamb's Head to Cod's Head	Ardgroom Cleandra Kilmakilloge	Mussels Mussels Mussels	A A B	
		Sneem/ Tahilla	Mussels	B	
		Coosmore All other Beds	Mussels Oysters	A B	
Bantry Bay	Ardnakinna Point to Fair Head and Lonehort Point to Bank Harbour Area bounded to the North by a line from Gortnakilla Pier to a point at 51° 37.5'N, 09° 42'W to Whiddy Point West to Relane Point. Sheep's Head to Black Ball Head	C'townbere South Shore All other Beds	Mussels Mussels Urchins Mussels	A B A B	Seasonal classification 1 Jan to 30 Jun, reverts to class B at other times (Note 1).
Dunmanus Bay	Sheep's Head to Three Castle Head	All Beds	Mussels Urchins	B A	
Roaringwater Bay Baltimore Harbour	Cousnaganniv Point to Frolic Point Barrack Point to Beacon Point and Lettuce Point to Spanish Point to	All beds All beds	Mussels Oysters	A B	

	Grig's Point				
Sherkin North	Licensed sites	All licensed Beds	Oysters	A	Seasonal classification 1 Mar to 30 Jun, reverts to class B at other times (Note 1).
Sherkin Kinish	Drawlaun Point to Long Point	All licensed Beds	Oysters	A	Seasonal classification 1 Dec to 31 May, reverts to class B at other times (Note 1).
Kinsale	Shronecan Point to Preghane Point	All Beds	Oysters	B	
Oysterhaven	Ballymacus Point to Kinure Point	All Beds	Oysters	B	
Cork Harbour	Between 8°16.4' W and 8° 15.6' W. Between 8°14.6'W and 8°13.2'W. Ahada Pier to Gold Point	North Channel West North Channel East Rostellan	Oysters Oysters Oysters	B B B	
Youghal Bay	Knockadoon Head to Knockaverry	All Beds	Oysters	B	
Dungarvan Bay	Helvick Head to Ballynacourty Point	All Beds	Oysters	B	
Waterford Harbour	Creadan Head to Hook Head	All Beds	Cockles Mussels Oysters Surf Clams *Spisula species)*	B B B B*	* Preliminary Classification (Note 2)
Bannow Bay	Ingard Point to Clammer's Point	All Beds	Oysters	B	
Ballyteigue Bay	Ballymadder Point to Crossfarnoge Point	All Beds	Oysters	B	

Wexford Harbour	Rosslare Point to The Raven Point	All Beds	Mussels	B	Preliminary classification (Note 2)
Malahide	Between 53° 25.4' N and 53° 29.4' N	All Beds	Razor Clams	B	
Skerries	Area bounded by a line from Hampton Cove to a point at 06° W, 53°36.3' N to a point at 06° W, 53°34.5'N to Shenick Island	All Beds	Razor Clams	A	
Gormanston / Laytown	Between 53° 38' N and 53° 40'N and Between 53° 41' N and 53° 42' N	All beds	Razor Clams	A	Seasonal classification 1 Jan to 31 Jul, reverts to class B at other times (Note 1).
Dundalk Bay	Area bounded to the East by 6 ° W, to the South by 53° 49' N and to the North by 54° N.	All Beds	Cockles Razor clams	B B*	 * Preliminary Classification (Note 2
Carlingford Lough (Irish Waters)	Ballagan Point to Cranfield Point and Rostrevor Quay to Greer's Quay	Ballagan Carlingford	Razor Clams Oysters Mussels Oysters	A A A A	

Lapsed classifications

The classifications for the areas listed below have lapsed because they are no longer active or because an insufficient number of samples were available for the review period.

Production Area Species

Traweenagh Mussels
Clew Bay (Newport Bay) Oysters
Kilrush Oysters
River Boyne Mussels

Notes

Note 1 Seasonal classifications
Where the data shows a clear seasonal trend over a number of seasons, different classification categories apply for different seasons. Details, where applicable, are given in column VI above.

Note 2 Preliminary classifications
Classifications are described as preliminary when an area is being classified for the first time or after a period in suspension. The term may also be used where an incomplete dataset of results was to hand.

Subsequent Re-Classifications since June 2008 are as follows;

a. Dunany, Dundalk Bay, Preliminary B for Razor Clams wef Sept 2008.
b. Clifden Outer, Preliminary B for Razor Clams wef Nov 2008.
c. Casheen Bay, Kilkieran, Preliminary A for Mussels wef Dec 2008.
d. Kilkieran Bay North, Preliminary A for Native Oysters wef Apr 2009.

Subsequent New Classifications since June 2008 are as follows;

a. Harry Lock Bay, Waterford Estuary, Preliminary B for Surf Clams.

The current classification listings of shellfish harvest areas are available on the SFPA website at http://www.sfpa.ie/ The website is updated throughout the year as new areas are classified or the status of existing areas is changed. The date assigned to the Classified Bivalve Production Areas in Ireland on the website indicates the most recent update.

Additional National Reference Laboratory activities in 2008

As well as assisting the competent authority in organising the national monitoring programme for classification the MI is also responsible for provided a virus testing service. To this end the MI has over the last four years introduced procedures for the analysis of Norovirus (NoV) and hepatitis A virus (HAV) in shellfish as these are the most commonly identified pathogens associated with shellfish consumption. These procedures are robust and reliable and have been used by a number of laboratories throughout Europe. However these procedures are not fully standardised internationally and this presents a barrier to routine virus testing and the introduction of internationally accepted standards. To address this, a European working group under the direction of the European committee for standardisation (CEN) was established to develop quantitative real-time PCR procedures to ISO standards. The MI is a participant in this working group and actively contributes to this process. The next step in this process is to undertake a large scale inter-laboratory validation in a number of laboratories throughout Europe which is planned for 2009. The Marine Institute is already successfully using the developed procedure and plans to apply for accreditation for NoV analysis with INAB in 2009. There are no immediate plans to introduce virus standards or routine monitoring in

Ireland or elsewhere. However the procedure as already been applied to shellfish sample implicated in outbreaks of illness, to provide additional quality assurance for exported shellfish and in number of small studies and surveys.
In October of 2008 the MI started a 3 year project funded by the EPA to investigate the reduction of NoVs during waste water treatment. This study will compare NoV survival during waste water treatment and in seawater alongside indicator organisms. In-situ studies will investigate NoV levels in influent and effluent from a waste water treatment plant. The relative impact of storm overflows and continuous treated sewage discharges will be investigated. Laboratory studies will also investigate the survival of NoV in seawater. Finally, laboratory-based studies will investigate the use of UV treatment of secondary effluents to reduce NoV levels.

The project has the following specific objectives

1. ***Quantify the level of norovirus found in sewage influent, intermediate stages and effluent in a secondary treatment WWTP and identify the extent of norovirus removal during sewage treatment.***

2. ***Determine the relative contribution of storm overflow discharges and continuous treated sewage inputs to norovirus contamination in shellfisheries.***

3. ***Establish the time required to reduce 90% of norovirus (T90 values) in seawater under typical winter and summer conditions.***

4. ***Determine the extent of the reduction of NoV levels using UV treatment.***

The first results from the monitoring phase of the project will be available in June this year.

Summary

- The SFPA is responsible for undertaking the microbiological monitoring programme and making classifications of shellfish harvesting areas in Ireland. The Marine Institute supports the SFPA in this role by organising analysis of sample, ensuring the quality of data generated and providing scientific advice as appropriate.

- In 2008, 1734 samples were taken for analysis for *E. coli* representing an increase of around 10% on the number of samples taken in 2007.

- The accuracy of the classification relies on the quality of the data generated. The SFPA and MI work together to ensure that sampling and analysis for classification purposes are undertaking to defined quality standards.

- A code of practice for microbiological monitoring was published in 2008 and the procedures identified in it were used fro the first time to classify shellfish harvesting areas in June 2008. Review of three years worth of monitoring data was used for the first time to decide on classifications 2008.

- Robust and reliable quantitative real-time PCR procedures for the detection of NoV and HAV are available in the MI and are being used for a number of roles. These procedures will undergo international validation through inter-laboratory trials with the ultimate aim of producing ISO approved procedures.

- Initial results from studies conducted by the Marine Institute on NoV reduction during waste water treatment will be available during the second half of 2009. Updates on the progress of the project will be available on the Marine Institute website under the shellfish microbiology section.

Azaspiracid Group - Scientific Opinion of the EFSA Panel on Contaminants in the Food chain

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Introduction

The European Food Safety Authority (EFSA) was set up in January 2002. It provides scientific advice on risks associated with the food chain.
In the European food safety system, risk assessment is done independently from risk management. EFSA is the "risk assessor" and in this role it produces scientific opinions and advice. These opinions and advice are then used by other EU institutions and member states to inform policies and legislation and as a foundation for taking effective and timely risk management decisions. EFSA's remit covers food and feed safety, nutrition, animal health and welfare, plant protection and plant health.

In their role as the EU's risk assessors, EFSA were asked to provide opinions on various shellfish biotoxins for which regulatory limits have been set in EU legislation. Specifically they were asked to assess the current EU limits with regard to human health and methods of analysis for various marine biotoxins as established in the EU legislation, including new emerging toxins, in particular in the light of:-

- the report of the Joint FAO/IOC/WHO ad hoc Expert Consultation on Biotoxins in Bivalve Molluscs (Oslo, September 26-30 2004), including the ARfDs and guidance levels proposed by the Expert Consultation;
- the conclusions of the Cdodex Committee on Fish and Fishery Product (CCFFP) working group held in Ottawa in April 2006;
- the publication of the report and recommendations of the joint European Centre for the Validation of Alternative Methods (ECVAM)/DG SANCO Workshop, January 2005;
- the report from Community Reference Laboratory (CRL) Working group on Toxicology in Cesenatico October 2005; and,
- any other scientific information of relevance for the assessment of the risk of marine biotoxins in shellfish for human health.

The subsequent opinion was produced by EFSA's Panel on Contaminants in the Food Chain.

Risk Assessment

The risk assessment was based on data provided from member states and other sources. Data on the prevalence of Azaspiracid in shellfish was derived from a total 12,270 samples, the results of which were provided to the Panel conducting the assessment (see table 1).

Of these 12,270 samples, 9,847 (80.25%) were provided from Ireland. The next highest contributing country was Norway with 1,851 (15.07%).

Table 1

Country	Year(s) of harvesting	Number of samples	Purpose of testing [a)]	Method of testing	LOD (µg/kg)	LOQ (µg/kg)
Germany	2005-2006	394	Pre- and Post-MC	LC-MS/MS	<1-10 [b)]	2-10 [b)]
Ireland	2003-2006	9,847	Pre-MC	LC-MS/MS	2	20
Norway	2004-2006	1,851	Pre-MC	LC-MS/MS	6	20
Spain [c)]	2005-2006	5	Pre-MC	LC-MS/MS	2	10
UK	2006-2007	178	Pre-MC	LC-MS/MS	2	5
Total		**12,275**				

(Source Alexander et al 2008)

A second critical factor in assessing the exposure of the population to AZA was the portion size consumed. A number of diet studies were examined which suggested a range of typical and exceptional portion sizes (see table 2).

Table 2

Country	Study	Number of consumers N (%)	Number of eating occasions for consumers /year	Mean portion weight (g)	95th percentile	Maximum portion weight (g)	Maximum frequency
France (7 days)	INCA 1999	- (11)	NA	10			NA
France (FFQ)	CALIPSO (bivalve molluscs)	962/997 (96)	NA	32	94	415	NA
France (FFQ)	CALIPSO (mussels)	862/997 (86)	NA	22	70	245	NA
Italy (7 days)	INN-CA 1994-96	212/1,981 (11)	47	83		1,000	4/week
Germany (7 days)	NVS 1985-88	150/23,239 (0.6)	171	107	400	1,500	3/week
UK (7 days)	NDNS 2000-01	212/1,631 (13)	51	114		239	4/week
The Netherlands (2 days)	DNFCS 1997-98	47/4,285 (1.1)	39	136	465	480	NA

(Source Alexander et al 2008)

In the analysis conducted by the FAO/IOC/WHO ad hoc Expert Consultation on Biotoxins in Bivalve Molluscs, a portion of 380g was used. This represented the 97.5th percentile from the studies used by that group.

For their analysis the EFSA Panel on Contaminants in the Food Chain opted for 400g as the 95th percentile from the data provided to them.

Exposure Assessment

In conducting the exposure assessment the Panel concluded, based on a probabilistic analysis, that consumption of a 400 g portion containing 40 µg/kg of AZA would result in an exposure of 16 µg AZA1 equivalents per person. This would be the equivalent to 0.25 µg/kg b.w. for 60 kg adult.

The figure of 40 µg/kg of AZA was used as this represented the 95th percentile of the AZA concentration from the negatively MBA tested samples in the data provided on the prevalence of Azaspiracid in shellfish (table 1).

Further, the Panel also calculated the chance of a specific exposure level being exceeded. In the case of 64 µg AZA1 equivalents per person the probability of exceedance was estimated to be 0.13% of and 2% in respect of 16 µg AZA1 equivalents per person.

In conducting the hazard characterisation, the Panel arrived at an acute reference dose (ARfD) of 0.2 µg AZA1 equivalents/kg b.w. The consequence of this figure was that in order for a 60 kg adult to avoid exceeding the ARfD, a 400 g portion of shellfish should not contain more than 12 µg AZA group toxins, i.e. 30 µg AZA1 equivalents/kg shellfish meat.

In furtherance of this thinking and conclusion it is possible that this figure of 30 µg AZA1 equivalents/kg shellfish meat could eventually be suggested as the new regulatory limit by the European Commission and / or member states when the time comes to formally consider EFSA's reply to the question originally submitted to them.

Discussion

The opinion of the EFSA Panel has been passed to the European Commission and it will be for them along with the member states to decide how to proceed.
While the Panel have been rigorous in their consideration of the issue, there are a number of areas that will possibly require further clarification, including:

- The portion size – 400g is, by any measure, a large portion of shellfish and an especially large portion of mussels given the size of the particular shellfish and the meat yield from them.

- Consumption Patterns – even if the 400g portion is considered to be a representative figure of the 95th percentile of consumption the issue of consumption patterns probably requires further investigation, as it is unclear whether it is relevant or was factored in that consumers ingest shellfish in these amounts on a regular occasion.

- Testing Methodologies – if regulatory limit was to be lowered it could render redundant the most used test for detecting AZA, namely the mouse bioassay.

> While this would be generally beneficial in respect of animal welfare it does introduce a new range of problems linked the to use of chemical testing. Testing for AZA is a competency that is, at the moment, the exclusive preserve of a few laboratories around Europe and widespread testing for this toxin using chemical methodologies may not be achievable in anything other than the long term.

The matter remains to be resolved and will be taken up by Ireland's representatives to the relevant European Commission Working Groups.

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Understanding the Shellfish Waters Directive

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Presented on behalf of the Department for the Environment, Heritage and Local Government

Background

The EC Shellfish Waters Directive (79/923/EEC) adopted on 30 October 1979 aims to protect or improve the quality of shellfish growing waters. The directive applies to bivalve molluscs and gastropods and sets out physical, chemical and microbiological water quality requirements that designated shellfish waters must comply with. In Ireland the directive was originally transposed in to law by Statutory Instrument (S.I.) 200 of 1994 in which 14 Shellfish waters were designated. This regulation was amended by S.I. No. 459 of 2001 which further established action programmes for each of the designated shellfisheries. The EC Shellfish Waters Directive (79/923/EEC) directive was repealed and replaced by a codified version 2006/113/EC from December 2006.

Following legal judgements from the European Court of Justice the Minister for Environment, Heritage and Local Government signed the European Communities (Quality of Shellfish Waters)(Amendment) Regulations 2009, Statutory Instrument No. 55 of 22009 on 10th February 2009. These regulations provided for the designation of an additional 49 Shellfish Waters bringing the total to 63. The shellfish waters sites currently designated can be viewed at the following website.

http://www.environ.ie/en/Environment/Water/WaterQuality/ShellfishWaterDirective/

Requirements of the Directive

The 2006/113/EC regulations require states to designate any shellfish growing waters requiring environmental protection. It lists 11 environmental quality parameters which must be monitored in designated areas (Table 1). Both Imperative (I) and Guideline (G) values may be given for each of the parameters monitored. Imperative standards must be attained whereas member states must "endeavour to observe" G values.
Member states must develop pollution reduction programmes which must demonstrate a plan to ensure no deterioration of water quality and where necessary improvement in designated areas. Pollution reduction plans are area specific and identify pollution pressures on designated shellfish water areas and identify specific remedial action where required.

Implementation of the Shellfish Waters Directive in 2009

On 10th February 2009 the government designated a further 49 shellfish waters bringing the total number of designated areas in Ireland to 63. Since November of 2008 the Department of Environment Heritage and Local Government (DEHLG) have been

responsible for the implementation of the Shellfish Waters Directive in Ireland. They have laid down a route map for the implementation of the directive. This plan and current progress is outlined in Table 2.

Action	Start Date	Finish Date
49 Additional sites selected for designation	2008	Completed
Identify Boundaries & Sampling Points for designated areas	2008	Completed
Consultation with stakeholders on site selection	2008	Completed
Scoping the Strategic Enivronmental Assessment (SEA)	2008	Completed
Production of SEA Reports	22/09/2008	Ongoing
SEA Consultation	1/11/2008	Ongoing
Make designations by regulation	2009	Completed
Establish Pollution Reduction Programmes	1/09/2008	Ongoing
Commence Testing Regime for Bays	1/10/200	Ongoing

Table 2. Planned Implementation of the SWD in Ireland

To assist with implementation a shellfish waters management committee has been established and operating since 2006. The committee recognises the need for cross departmental working to effectively implement the regulations and is made up of representative from the following organisations:

- Dept. Environment Heritage & Local Government
- Dept. Finance
- Dept. Agriculture & Food
- Dept. Community Rural & Gaeltacht affairs
- EPA / BIM / Marine Institute
- City & County Managers Assoc in sub group

The committee has been tasked with the following duties:

- Reviewing progress in relation to existing action programmes

- Advising the Minister when additional actions are required, the authority responsible and proposed timeframes
- Reviewing the ongoing sampling and analysis programme
- Developing and overseeing action programmes in respect of additional areas to be designated as shellfish waters

Monitoring programmes for all the shellfish areas have now been developed by the Marine Institute and have commenced sampling with the support of the SFPA in all areas. Data from the monitoring programmes will be reviewed against compliance levels and assessments made which will feed into the pollution reduction plans.
A significant change introduced in Statutory Instrument No. 55 of 22009 is that that clear responsibilities are placed on local authorities to protect and improve water quality at designated sites for parameters of water quality in the Directive.

Summary

- The Shellfish Waters Directive requires shellfish growing areas to be designated. Once designated water quality in areas must be protected or if required improved as judged by standards for a suite of environmental standards.
- DEHGL is responsible for the implementation of the directive in Ireland.
- Sixty three shellfish growing waters are now designated in Ireland under the Shellfish Waters Directive.
- Monitoring programmes have been established for all shellfish areas and sampling commenced in November.
- Pollution Reduction Plans are being established for each of the designated shellfish growing areas. Results from the monitoring programmes will feed into the pollution reduction plans and inform decision making during that process.

Parameter	Frequency of monitoring	Imperative (I) standard		Guideline (G) Value	
pH	Quarterly	pH between 7 and 9			
Temperature	Quarterly			Must not exceed by 2ºC temperatures found in waters not affected	
Coloration	Quarterly	must not deviate by 10 milligrams per litre over waters not affected by discharges			
Suspended Solids	Quarterly	Must exceed by more than 30% suspended solids present in waters not affected by discharges			
Salinity	Monthly	<40 practical salinity units		12-38 practical salinity units	
Dissolved Oxygen	Monthly	>70%		≥ 80% (average value)	
Hydrocarbons	Quarterly	Must not be present in quantities that will produce a visible film or be harmful to shellfish			
Organohalogens	Half-yearly	Polychlorinated Biphenyls 0.3 µg.litre^{-1}		Polychlorinated Biphenyls 100 µgKg^{-1} shellfish	
Metals	Half-yearly	µg.litre^{1} seawater	Aresenic 40 Cadnium 540 Chromium 30 Copper 10 Lead 20µ Mercury 0.4 Nickel 50 Silver 10 Zinc 200	mg Kg^{-1} Shellfish flesh	Aresenic 30 Cadnium 5 Chromium 6 Copper 400 Lead 7.5 Mercury 1 Nicke l 5 Silver 15 Zinc 4000
Faecal coliforms	Quarterly			≤300 faecal coliforms 100g^{-1} shellfish flesh	
Substances Affecting Taste	If presence presumed	Limited so taste is not impaired			

Table 1. Parameters and standards in statutory instrument No. 268 of 2006

ASTOX 2 The Biological Source, Chemical and Toxicological Studies on Azaspiracids.

Conor Duffy[1], Dr Philipp Hess[2].
[1]Marine Institute, Oranmore, Galway
[2]IFREMER, Nantes, France.

Introduction:
Azaspiracids (AZA) are a marine toxin group first discovered in Ireland. In November 1995 mussels cultivated in Killary Harbour, on the west coast of Ireland were implicated in the poisoning of at least 8 people in the Netherlands (McMahon and Silke, 1996). Symptoms resembling those of the known Diarrheic Shellfish Poisoning (DSP) were reported in individuals. Subsequently it was shown that the cause of the illness were toxins later named as Azaspiracid (AZA) (Satake et al., 1998; Ofuji et al., 1999).

There are numerous analogues of azaspiracids, either produced by microalgae or as a result of metabolism in shellfish. At least 20 analogues of AZA were reported by Rehmann et al., 2008 in naturally contaminated shellfish. Of the analogues AZA-1. AZA-2, and AZA-3 are considered to contribute most to overall toxicity as the concentration levels of the remaining analogues were found to be less than 5% of total AZA equivalence and thus are unlikely to contribute significantly to overall toxicity (Rehmann et al., 2008).

Azaspiracids are regulated within the European Union in accordance with regulation (EC) No 854/2004. Bivalve molluscs must not contain AZA in the whole body or any part edible separately that exceed the regulatory limit of 160 µg of azaspiracid equivalents per kilogram. AZA equivalent concentration is determined based on the sum of the concentration of AZA-1, AZA-2, AZA-3 converted to equivalent concentration based on the published toxic equivalence factors (TEF).

The mouse bioassay (MBA) is the official test for the analysis of molluscs for DSP and AZP toxins in Europe. Alternative method may be used once standards are available and the methods are validated following an internationally agreed protocol (Commission Regulation EC/2074/2005). Liquid Chromatography Mass Spectrometry (LCMS) hold numerous advantages over the MBA but the validation of alternative methods such as LCMSMS has been hampered by the lack of availability of standards and certified reference material.

The ASTOX Projects

ASTOX (Hess et al, 2007), Isolation and purification of azaspiracids from naturally contaminated materials and evaluation of their toxicological effects, commenced in January 2003 to address the need for analytical methods and quality control tools for the analysis of toxins in shellfish and to carry out initial toxicology assessment of azaspiracids. In order to obtain material for preparation of standards, reference material and for toxicology studies, AZAs and DTX-2 were isolated for contaminated shellfish and characterised. ASTOX lead to the wide spread availability of standards which has advanced the development of analytical method which in turn has fostered wider research in the area of azaspiracids and improved national monitoring programmes. The initial in-vitro toxicology work carried out on mammalian cell lines and functional assays identified initial pathways of

action of AZA. Intraperitoneal injection of mice also lead to the establishment of a robust Toxic Equivalent Factor for DTX-2. The analytical techniques for isolation and analysis of toxins developed in ASTOX will enable the production of larger quantities of purified material for longer term in-vivo exposure experiments in ASTOX2.

ASTOX2, **The Biological Source, Chemical and Toxicological Studies on Azaspiracids** is funded by the Marine Institute under the Marine Research Sub-Programme of the National Development Plan 2007 – 2013. Scientists in the MI recognised the need to further this area of research which is important to the industry, monitoring, and regulatory bodies. A proposal was submitted and was successful in securing funding for a three year study to be completed between 2009-2011.
This project aims to strengthen the existing national biotoxin monitoring programme for shellfish toxins through

(i) the elucidation of the source organism of azaspiracids;
(ii) the clarification of relative and combined toxicities and the mode of action of analogues of azaspiracids and other lipophilic toxins; and
(iii) the sustainable supply of AZA-calibrants in support of international efforts in the validation of quantitative test methods that can be used to refine, reduce and replace animal testing.

There are seven internationally recognised project partners, who will participate in this research project under their respective areas of expertise.

- Marine Institute, Ireland
- Dublin Institute of Technology, Ireland
- Norwegian School of Veterinary Science, Norway
- National Oceanic & Atmospheric Administration, USA
- National Research Council, Canada
- Alfred Wegener Institute, Germany
- IFREMER, France

Studies on the causative organism will provide insights into the ecological fate of azaspiracids in the marine food web. The project is designed to address the trophic transfer of AZAs through the marine foodweb. Although an AZA-producing organism, *Azadinium spinosum*, has been isolated from North Sea waters by scientists from Alfred Wegener Institute (AWI), at this stage, it cannot be excluded that there is more than one biogenic source of AZAs, as has been demonstrated for saxitoxin and its analogues, which are produced by at least 3 dinoflagellate genera (*Alexandrium spp., Gymnodinium catenatum and Pyrodinium bahamense*) and also by a number of *cyanobacteria*. It is expected that one of the outcomes of the project is in-depth knowledge of one or several of the primary causative organism(s) or at least of near ecological neighbours of the primary biological source(s) in Irish waters. A further output of the project is knowledge on the mechanisms of transport of AZAs in the marine environment. The techniques of passive sampling developed by the MI during the EU-FP6-project BIOTOX will aid in these studies, as well as the laboratory studies of transfer of toxin through culturing of toxic prey and heterotrophic organisms. The overall outcome of this part of the project will thus be the increased knowledge on the biological source(s) and mechanisms of transport of AZAs in the marine environment, knowledge that will provide an input into developing a strategy to more effectively monitor, and eventually predict, the occurrence of AZAs in shellfish.

The main outcomes of this project anticipated in the area of chemical support are materials to be used in the toxicology part. Such materials will include:

- mg-quantities of AZA1, -2 and –3, sufficiently pure and characterised to be used in toxicology studies.
- shellfish homogenates sufficiently well characterised in terms of homogeneity and stability to be used in toxicology studies
- mouse feed homogenates sufficiently well characterised in terms of homogeneity and stability to be used in toxicology studies

Further outputs from the chemical support activities will also include:

- in-house validated methods to analyse phytoplankton and shellfish for a variety of AZA-analogues (more than 20 of which have already been identified in shellfish)
- in-house validated methods to analyse mammalian tissues and mouse feed for such AZA-analogues

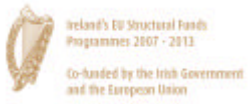
It is not the aim of this project to formally validate analytical methods for official control of shellfish toxins in shellfish. The aim of the project is to descriptively validate methods to ensure that performance characteristics of methods are sufficient to conclusively interpret results from analyses of phytoplankton cultures, animal tissues and matrices fed to mammals during the toxicology trials.

The **studies in toxicology** are aimed at answering four major questions:

- which is or are the molecular target(s) of AZAs in mammalian cells, i.e. the mode of action at molecular level
- what is the pharmacokinetic behaviour of AZAs
- what are the relative toxicities of AZAs
- what are the possible consequences of the co-occurrence of AZAs with other lipophilic shellfish toxins.

In vitro studies will follow the generic approach developed in the previous NDP-project (ASTOX), i.e. to investigate the possible pathways of action outlined from the gene-chip studies by the groups of Doucette and Ryan as well as the observations at molecular level by the groups of Rossini and Botana. The pharmacokinetic behaviour of AZAs will be studied in animals (mice and pigs) to verify previous *in vitro* studies and to elucidate the possibility of transfer across the intestinal barrier. It is the lack of knowledge regards the transfer across the intestinal barrier that results in considerable concern about the toxic effects observed in oral studies in mice by Ito et al. (2000 and 2002). The European Food Safety Authority is presently re-evaluating the current EU regulation on AZA group toxins, based on the most recent risk assessment by the Irish Food Safety Authority (2006), and available information on toxicokinetics, toxicodynamics and epidemiology.
Ultimately, the outcome of the project may lead to revision of the currently prescribed regulatory limits for AZAs in Europe.

This project (Grant-Aid Agreement No. PBA-AF-08-001) is carried out under the *Sea Change* strategy with the support of the Marine Institute and the Marine Research Sub-programme of the National Development Plan 2007–2013, co-financed under the European Regional Development Fund.



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Molecular Methods For Monitoring Harmful Algal Bloom Species.

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EU SPIES DETOX project collaborators : Nicolas Touzet[1], Robin Raine[1], Elizabeth Turrell[3], Catherine Collins[3] & Jennifer Graham[3] [1] NUI, Galway, [3] Marine Scotland.

Introduction:

Shellfish production worth approximately €60 million to the Irish economy in 2006 (Browne et al., 2007) and several €100 millions to other European countries can be adversely affected by the presence of harmful microalgae (HABs). Toxins produced by *Dinophysis, Alexandrium and Pseudo-nitzschia* species can accumulate in shellfish and have the potential to cause serious human illness. In order to satisfy EU legislative requirements pertaining to the production and export of shellfish (EC Hygiene Regulations 2004, No. 853/2004 and No. 854/2004, which replaced the EU Shellfish Hygiene Directive 91/492/EEC in January 2006), monitoring the presence of harmful algal species and biotoxins in coastal waters is performed by EU member states.

Routine microscopic monitoring methods are unable to identify certain toxic species, in particular, *Alexandrium and Pseudo-nitzschia spp.* Electron microscopy is required for species identification and this technique cannot be integrated into a routine monitoring programme.

Molecular techniques utilise unique sequence signatures within microorganism genomes for species specific identification. Molecular methods applied for the identification and quantification of HAB species include Fluorescent in-situ hybridisation (FISH) and in-vitro amplification based methods, in particular, real-time PCR.

Rationale and benefit to the aquaculture industry:

Molecular methods identify toxic HAB species based on unique sequence signatures found mainly in ribosomal genes (rRNA) and internally transcribed spacer regions (ITS) in ribosomal gene operons. These methods can compliment or replace existing monitoring programme methods by providing species identification rapidly for large numbers of samples. The results of these analyses have the potential to be combined with information obtained from "at site" monitoring of shellfish waters for biotoxins providing an "early warning" to shellfish producers. Additionally, the methods can be applied as research tools to help understand bloom dynamics.
In the Phytotest project, a collaboration between the National Diagnostics Centre at NUI, Galway and the Marine Institute, a panel of real-time PCR tests were designed to detect and identify *Dinophysis spp.* and selected *Pseudo-nitzschia spp*. found in Irish waters. This project, the tests developed and their application in monitoring were described by Kavanagh et al. (2008) in the "Proceedings of the 7th Shellfish Safety Workshop". The tests developed in this project are being used by the Marine Institute to support the current monitoring programme.

The EU SPIES DETOX project is a 3-year EU funded project which commenced in 2006 where research performers are working with the shellfish industry in Ireland, Spain, Scotland and Norway to develop methods to improve monitoring programmes and improve the detoxification of shellfish. In the EU SPIES project, researchers in the Molecular Diagnostics Research group at NUI, Galway are working with researchers at Marine Scotland in Aberdeen, Scotland to develop real-time PCR tests for other important *Pseudo-nitzschia species* and FISH probes and real-time PCR tests for *Alexandrium spp.* In parallel, workpackages 1-2 are investigating the feasibility of using selected resins deployed as "solid phase adsorption toxin tracking" (SPATT) devices to provide an early warning system for biotoxins in shellfish production waters. An overview of the EU SPIES DETOX project has been provided in these proceedings by Dr. Elizabeth Turrell, Marine Scotland.

Design and application of molecular methods:

The development of species-specific FISH probes or real-time PCR tests begins with culturing of the target HAB species and closely related species, extraction of nucleic acid from these species and DNA sequencing of the rDNA or ITS regions. For FISH, a DNA probe targeting a unique region of the rRNA is designed and coupled with a fluorescent tag that acts as a reporter for the probe. The water sample containing HABs is tested with the FISH probe by fixing the water sample to a membrane and treating it to allow the probe pass through the cell wall and hybridise to the specific rRNA sequence identifier within the HAB species cell. After washing to remove unbound probe, the sample is viewed under a fluorescence microscope and samples which are positive for the HAB species will fluoresce (Figure 1). For real-time PCR, PCR primers and a DNA probe are designed and configured into a real-time PCR test. Water samples are tested for HABs species following filtration of the water sample (25ml) and nucleic acid (DNA) extraction. DNA is added to the real-time PCR reaction and a characteristic fluorescent diagnostic signal is obtained for positive samples.

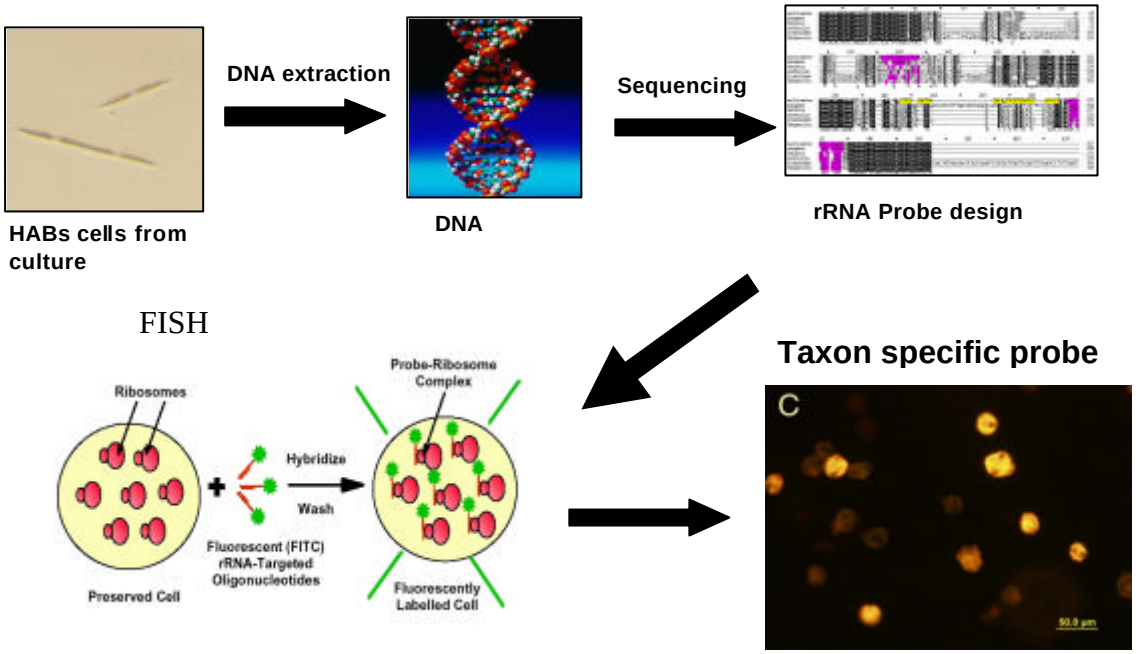

Figure 1: **Schematic representation of the steps involved in design and application of Fluorescent *in-situ* hybridisation (FISH) probes.**

Molecular methods for Alexandrium spp. identification:

At NUI, Galway, FISH probes have been developed for 7 Alexandrium species based on the ribosomal gene targets (rRNA), large ribosomal subunit (LSU) and small ribosomal subunit (SSU). Whole cell FISH (WC-FISH) has been optimised to enable 2 probes labelled with different fluorescent reporters to be used in combination for the simultaneous identification of 2 *Alexandrium* species in environmental samples (Figure 2). Additionally, the method has been optimised so that calcofluor is added to the test enabling the genotypic identification of the *Alexandrium* to species level with the FISH probes and the phenotypic confirmation of the species based on morphology using calcofluor (Touzet and Raine, 2007). WC-FISH probes for *A. minutum GC, A. tamarense* WE *and A. tamarense* NA have been tested in field samples collected from Cork Harbour, Ireland and from Shetland and Orkney, Scotland in 2007 and Cork Harbour 2008. Samples from Cork Harbour were tested with the dual WC-FISH test (*A. minutum GC and A. tamarense* WE) by morphotaxonomy using calcofluor and by qPCR with the *A. minutum* GC real-time PCR test. WC-FISH identified high numbers of *A. minutum* GC in the samples compared to *A. tamarense* WE. When compared with morphotaxonomy using calcofluor (standard method) for enumeration of the target species, WC-FISH under-estimated the *A. minutum GC and A. tamarense* WE cells by a factor of 1.2.

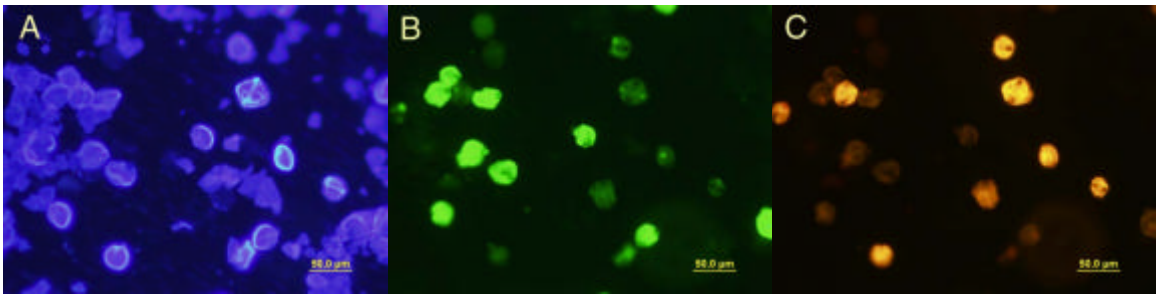

Figure 2**: Dual WC-FISH for *A. tamarense* WE and NA. A. Calcofluor staining; B. *A. tamarense* WE (FITC labeled probe); C. *A. tamarense* NA (CY3 labeled probe).**

At Marine Scotland and NUIG, real-time qPCR tests have been designed for a range of *Alexandrium spp. (A. tamarense WE, A. tamarense NA, A. minutum NH, A. minutum GC, A. ostenfeldii, Alexandrium genus)* incorporating TaqMan or Hybridization probe (HybProbe) fluorescent detection chemistries and targeting either the rDNA LSU or ITS1-5.8S genomic regions in *Alexandrium*. Figure 3 shows an example of the *A. minutum* GC real-time PCR test incorporating Hybprobes showing the detection of 3 strains of *A. minutum* GC by quantification curves and melt-peak analysis. The tests were determined to be specific with selected specificity panels and the limits of detection of the tests (LODs) were established reproducibly in independent experiments at approximately 1 cell or less equivalents for all tests.

Figure 3:

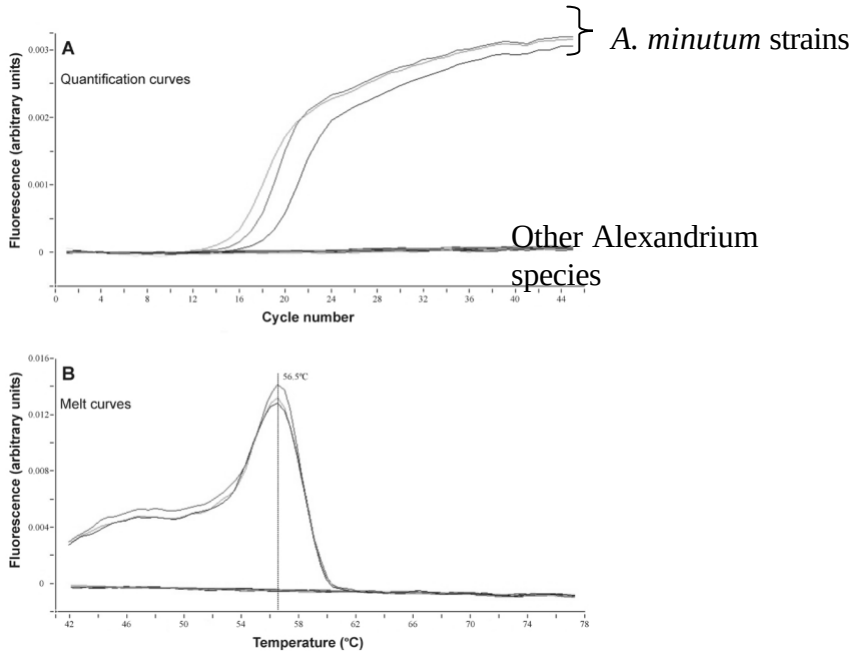

Figure 3: Real-Time PCR (A) quantification curves and (B) melt-curves showing the specificity of the *A. minutum* (GC) test tested against a panel of Alexandrium and dinoflagellate species. In this test run, the 3 *A. minutum* strains tested were detected while a range of *Alexandrium spp.* and other spp. were not detected in the test. \

The per test for *A. minutum* GC was evaluated on wild samples collected from Cork Harbour in 2007 and 2008. Quantification of the *A. minutum* GC by qPCR was compared with enumeration by WC-FISH and morphotaxonomy. Linear regression analyses were carried out to examine the distributions of the *A. minutum* concentrations derived using the three quantification methods. Positive relationships were observed between the methods. In 2007, qPCR overestimated the concentration of *A. minutum* GC compared to morphotaxonomy while in 2008 qPCR underestimated the **concentration of *A. minutum* GC** compared to the reference method (Touzet et al., 2009). Testing of field samples from Scottish waters is ongoing

Molecular Methods for *Pseudo-nitzschia spp.* identification:

As part of the EU SPIES DETOX project real-time PCR tests were developed to identify *P. seriata and P. multiseries.* These tests target the rRNA ITS1 region in *Pseudo-nitzschia spp.* and incorporate hybridization probe technologies. Specificity of each test has been verified using a broad panel of indigenous non-target phytoplankton species. Figure 4 shows the specificity of the *P. seriata* test as demonstrated by a *P. seriata* species-specific melt peak at 56°C.

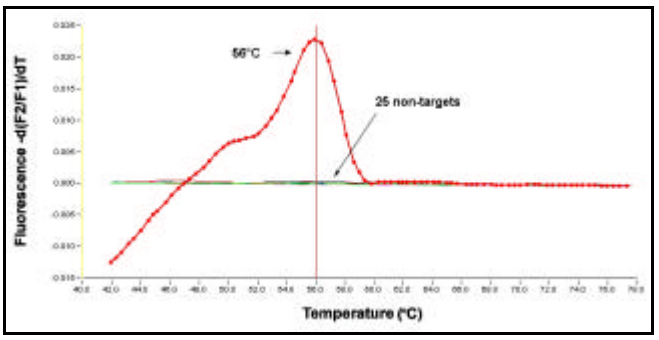

Figure 4: **Real-time PCR melt-peak at 56°C for the *P. seriata* specific test.**

The limits of detection of the P. seriata and P. multiseries real-time PCR tests are 1-10 cell equivalents. Figure 5 shows the quantification curves obtained when testing DNA from P. seriata cells in the range 10,000 – 10 cells.

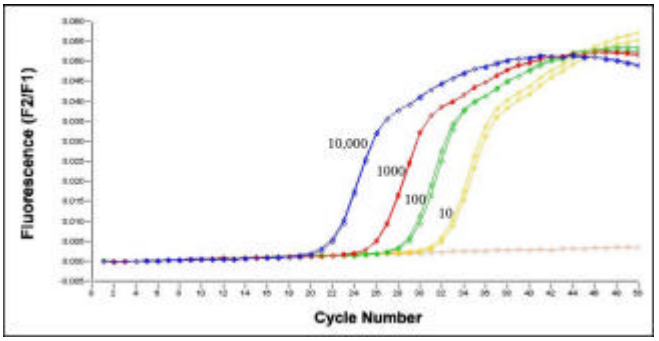

Figure 5: Real-Time PCR quantification curves for the P. seriata specific test testing DNA from cells in the range 10,000 – 10 cells.

Testing of water samples (25ml) is performed on DNA extracted from filtered samples using a combination of freeze-thawing and chemical extraction. Real-time PCR tests for *Pseudo nitzschia spp* -. including *P. australis, P. fraudulenta, P. pungens and P. delicatissima* (Phytotest project) and *P. seriata and P. multiseries* (EU SPIES DETOX project) and *Dinophysis spp. D. acuta and D. acuminata* (Phytotest project) have been evaluated on field samples collected between May and August 2008 from Killary Harbour, Clew Bay and Cork Harbour. Sixty-two weekly water samples (25 ml) were analysed by light microscopy for the presence of *Pseudo-nitzschia spp. –P. seriata and P. delicatissima* type cells and *Dinophysis spp.* cells. *P. seriata* type cells were present in 87% of samples while *P. delicatissima* type cells were observed in 64 % of samples.

D. acuta and D. acuminata were observed in 5 % and 21 % of samples respectively. The real-time PCR tests identified *P. seriata, P. multiseries, P. australis, P. fraudulenta, P. delicatissima and P. pungens* in 24%, 30%, 24%, 32%, 0% and 22% of samples respectively. *D. acuta and D. acuminata* were detected in 19% and 1% of samples respectively. Further evaluation of these tests is planned for 2009.

Conclusions:

Molecular methods have been developed to identify *Dinophysis, Alexandrium and Pseudo-nitzschia* species. Their application for monitoring water samples for these species has been demonstrated. These methods are rapid and can handle large sample numbers. They provide important information to monitoring programmes to help inform the decision making regarding the safety of shellfish production waters.

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AN OVERVIEW OF THE EC 6th FRAMEWORK PROJECT SPIES-DETOX

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Introduction

SPIES-DETOX, a three-year FP6 Collective Research project, is a strategic collaboration between Government Departments, leading National Universities, Research Laboratories, Small to Medium Enterprises (SMEs) and Industry Association.

SPIES

- To work with the aquaculture industry to develop 'early-warning' tools for the detection of toxic algae and toxins before shellfish are contaminated

DETOX

- To develop procedures, for use by industry, to remove algal toxins from contaminated shellfish and aid the implementation of HACCP systems

Groupings (IAGs). By working together the project has the following ambitions:

Work-packages

The aims are realised through progression of six research and training work-packages. A summary of progress to date against each work-package and hopes for the future are provided.

Work-package 1: To investigate new methods using solid phase adsorption toxin tracking (SPATT) for detection of algal toxins (amnesic shellfish poisoning (ASP), paralytic shellfish poisoning (PSP) diarrhetic shellfish poisoning (DSP) toxins and other marine lipophilic toxins) in the water column; this may serve as an early shellfish toxin contamination warning mechanism for areas important to shellfish harvesting

SPATT was previously proposed, by a New Zealand research team (Mackenzie et al. 2004), as a tool to facilitate monitoring of lipophilic shellfish toxins (LSTs) in shellfish harvesting areas. SPATT was founded on the observation that when low levels of toxic phytoplankton

are present in the water column significant amounts of toxins are present in seawater. It was considered that a lag between detection of extracellular toxins adsorbed onto porous synthetic resin, phytoplankton peak cell densities and highest toxin concentrations in shellfish can be observed, providing an early warning of potential harmful algal events.

During SPIES-DETOX, protocols (resin type, type and volume of solvent extraction) for SPATT of LSTs were optimised using SEPABEADS®SP700 as the adsorbent. Subsequently, SP700 was assessed for the adsorption and desorption of an additional potent toxin group; the spirolides (SPXs). A range of adsorbents were also evaluated to determine if they could be applied to SPATT for hydrophilic phycotoxins including domoic acid (DA), associated with ASP, and a wide range of PSP toxins.

Using laboratory-scale experiments, the uptake and desorption of DA and PSP toxins by candidate adsorbents from seawater was investigated. The best adsorbent for DA was found to be Amberlite® XAD761 which demonstrated, on occasion, nearly 100 % binding of available DA. A computationally designed polymer (CDP) based on ethylene glycol methacrylate phosphate (EGMP) was able to adsorb a wide range of PSP toxins. The adsorption behaviour of PSP toxins was further assessed using cultures of *Alexandrium tamarense*. The EGMP polymer accumulated *neosaxitoxin* (NEO), *saxitoxin* (STX) *gonyautoxins* (GTX) 1 to 4, and C toxins with differences in adsorption and equilibrium rate.

The SPIES-DETOX project now aims to validate the use of these adsorbents for use in toxin monitoring programmes and assess their potential as an 'early warning' technology for the aquaculture industry. Field studies were undertaken at aquaculture sites in Scottish, Irish, Norwegian, Spanish, and Greek waters. Optimised SPATT bags, containing SP700 or XAD761 as the adsorbent, were deployed by aquaculturists in conjunction with sampling of phytoplankton, local bivalve shellfish and/or routine regulatory monitoring at some sites. SP700 extracts were analysed using multi-toxin LC-MS analysis for LSTs and SPXs and by more rapid, 'field friendly' methods (ie. the Toxiline-DSP kit and DSP-Check ELISA). Amberlite extracts were examined using HPLC, LC-MS or the rapid Biosense-ASP ELISA test kit.

Using LC-MS, okadaic acid (OA), dinophysistoxins (DTXs), pectenotoxins (PTXs), azaspiracids (AZAs), yessotoxins (YTXs) and SPXs were detected in SP700 extracts. DA was detected in XAD761 extracts. Occurrence of different toxins was site dependent and changes in toxin profiles and concentrations were observed at all sites during the sampling period. Changes mostly appeared to be related to changes in the phytoplankton community structure from which the toxins originate. For example, Figure 1 shows concurrent detection of a SPX and the causative dinoflagellate, *Alexandrium ostenfeldii* in Cork Harbour, Ireland. Figure 2 shows detection of DA, the presence of potentially causative *Pseudo-nitzschia spp.* and DA in mussels at Loch Ewe, Scotland.

Rapid test kits were useful for detection of DA from XAD761 extracts and for detection of OA and DTXs from SP700. Results revealed higher DSP toxin concentrations when using the Toxiline-DSP kit compared with results obtained using LC-MS. However, the pattern of toxin adsorption of the resins during the monitoring periods were similar to those of LC-MS suggesting use of the kit could reduce analysis costs and provide a simple, sensitive system that could be used by shellfish harvest managers.

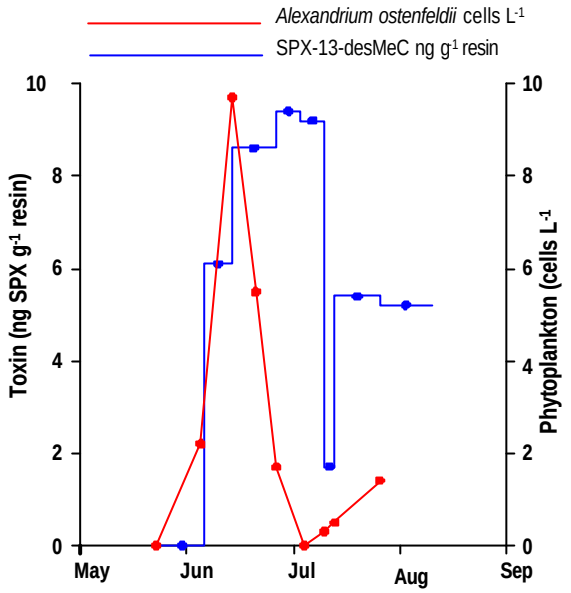

Figure 1. Spirolide-13-desMeC detected in SPATT during 2007 relative to *A. ostenfeldii* cell numbers observed using molecular probes

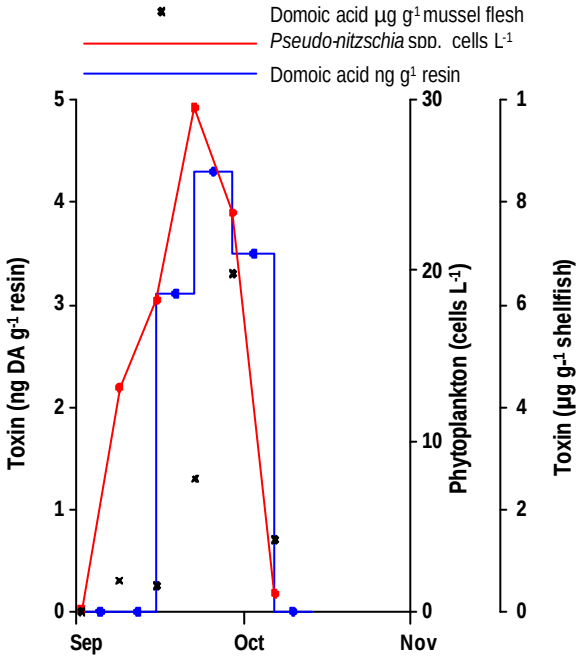

Figure 2. Domoic acid detected in SPATT during 2008 relative to *Pseudo-nitzschia* cell numbers and domic acid detected in mussels

AZAs were not detected in SPATT sachets before or immediately after highest peak cell densities of *Protoperidinium spp.* were observed (Fig. 3). The causative organism of AZAs was previously recorded as *Protoperidinium crassipes*, although doubt existed within the scientific community on whether this dinoflagellate species was the main progenitor of this group of toxins. Recently, a small photosynthetic thecate dinoflagellate (*Azadineum spinosum)* isolated from the North Sea has been identified as a producer of AZAs (Tillman et al., 2009). Difficulties in observing this small dinoflagellate using routine light microscopy as well as toxin transfer and transformation in food webs may allow SPATT to provide a better indicator of possible shellfish contamination with AZAs than phytoplankton monitoring.

Overall, these preliminary results suggest that toxin concentrations determined from SPATT extracts have potential to be used to set action levels that may be employed by the

aquaculture industry as an 'early warning' technology to predict closure and by regulatory authorities as criteria for the opening and closure of harvesting areas – although a longer time series of data at each site will be required to assess this possibility.

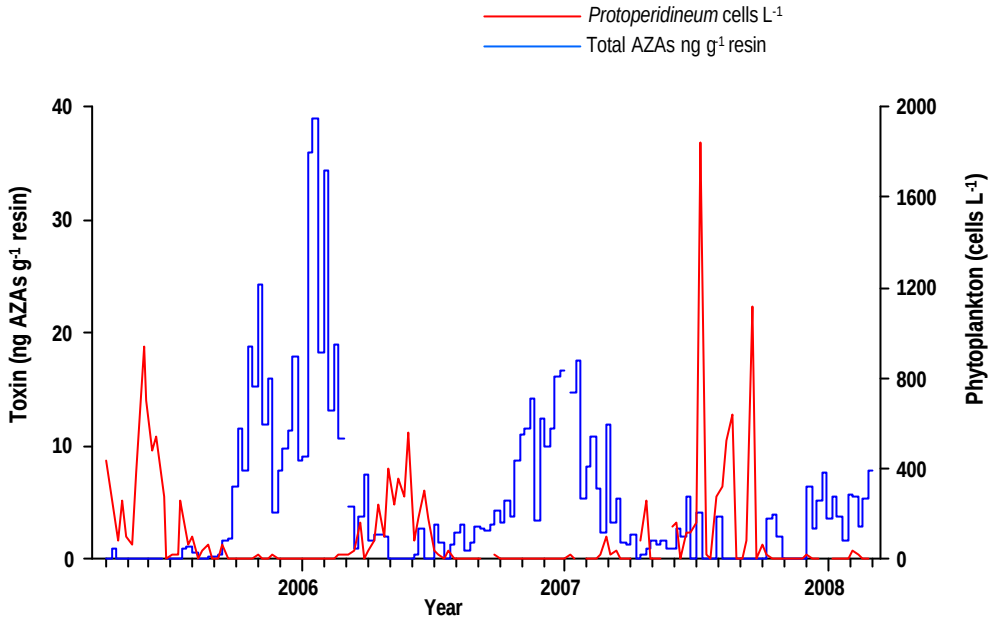

Figure 3. Azaspiracids; AZA-1, AZA-2 and AZA-3 in SPATT relative to dinoflagellate cell numbers

Work-packages 2 & 3: To develop new techniques to combine SPATT detection methods in remote samplers and the use of molecular techniques for the identification of toxic algal species - leading to rapid methods of detection of specific species of toxic phytoplankton

Ideally, the potential impacts of toxic phytoplankton should be assessed with integrated detection of the causative phytoplankton and toxins using *in situ* sensors in real or near-real time. As a first stage, the SPIES-DETOX project is using an auto-sampler (Aqua Monitor). The auto-sampler holds adsorbent resins that can be flushed with water and subsequently analysed to determine adsorbed toxin concentrations and profiles. Concurrently, the auto-sampler automatically captures water samples for phytoplankton detection and identification using molecular methods (WC-FISH probes and real time-PCR assays)

Following laboratory testing, an auto-sampler was deployed in Loch Ewe, Scotland (Fig. 4) and preliminary results demonstrate such equipment could aid in providing estimates of toxin release in the open sea. Further deployments in Ireland (Killary Harbour and Clew Bay, Ireland) and Scotland are now planned for summer 2009.

Molecular methods for identification of toxic algal species have advantages over traditional microscopic methods. Many toxic species cannot be identified accurately using light microscopy and require sophisticated scanning or transmission electron microscopy for definitive identification. Molecular methods are rapid, can identify toxic species based on species-specific genetic signatures and they are amenable to high-throughput analysis. Therefore, molecular methods can be used both for monitoring and as research tools.

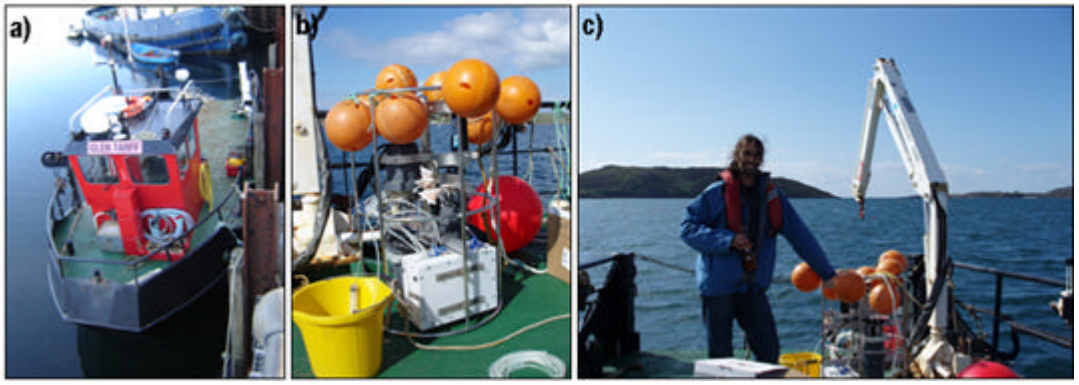

Figure 4.

Deployment of the Aqua Monitor at Loch Ewe - a) Work boat (belonging to shellfish aquaculturist) used to deploy the Aqua Monitor, b) Aqua Monitor (with floats) on deck prior to deployment, c) Crane used to hoist the Aqua Monitor

Development of molecular methods including WC-FISH and real-time qualitative and quantitative PCR methods for identification of important *Alexandrium* and *Pseudo-nitzschia species* commenced in the first phase of the SPIES-DETOX project. The application of these methods for the identification of toxic phytoplankton in wild samples has now been demonstrated and a detailed account for Irish waters is provided by Evelyn Keady and Majella Maher in these proceedings. The molecular assays will be validated and where relevant, results will be correlated with the toxin profiles obtained from the SPATT bags and auto-sampler deployed in Irish and Scottish waters

Work-package 4: To investigate the use of different protocols to wash ASP (and other algal) toxins from contaminated shellfish during processing

It has been proposed that the risks from eating raw shellfish products that have been contaminated with algal toxins can be reduced. Procedures for safe 'shucking' and washing of scallops contaminated with the ASP toxin, DA, were previously developed in the EC project, TALISMAN. It was demonstrated that DA, can be eliminated by washing to such an extent, that even in highly contaminated scallops, all edible parts should have DA toxin concentrations below the regulatory safety limit (Fig. 5). These results suggest edible scallop tissues need not fail 'end-product testing' for DA. Subsequently, scallop processors in Scotland were provided with Food Standards Agency Scotland training on practicalities of processing, correct 'shucking', washing and 'end-product testing' combined with HACCP systems. Currently shucked scallops from approved processors do not appear to be failing end product testing for DA.

During SPIES-DETOX, the washing and shucking protocols were further validated and may provide shellfish restraunteurs with rapid methods for selling scallop products. Unfortunately, washing protocols do not decrease PSP toxins to the same extent as DA and an alternative approach is being developed in Work-package 5.

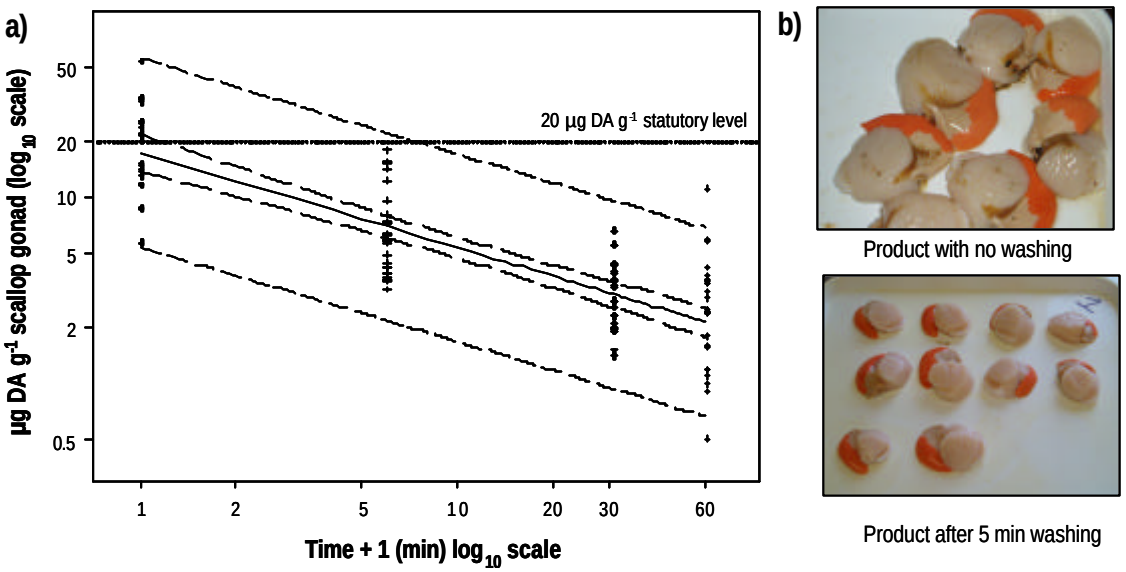

Figure 5. a) DA concentration (wet weight) in gonads of king scallops (n = 80) washed for 0, 5, 30 & 60 min. A Regression line (—), 99% upper and lower confidence limits for the mean (---), 99% upper and lower prediction limits for single values (– – –) and the 20 µg DA g^1 statutory level are given. b) Washing did not cause the product to deteriorate. Work-package 5: To investigate the bacterial degradation of algal toxins and the use of microencapsulated bacteria to purify these toxins from commercially important shellfish researchers have previously isolated marine bacteria which are capable of growing on PSP and ASP toxins as a sole carbon source and which may be able to metabolise complex groups of algal toxins (Stewart et al., 1998; Smith et al, 2001; Donovan et al., 2008).

It is considered that these bacteria must be able to degrade algal toxins *in vivo* and could play a significant part in toxin elimination in shellfish; as such possible practical applications are feasible in terms of manipulating the bacterial flora of shellfish to depurate shellfish of these toxins.

Results from SPIES-DETOX suggest that an array of bacteria isolated from shellfish, phytoplankton and seawater will have the capacity to degrade and/or biotransform PSP, ASP and DSP toxins. Candidate bacteria have been identified and are now being grown with different concentrations of algal toxins - degradation of the toxins and any changes in toxin profiles are monitored over time. The next stage is to develop a practical method of detoxifying cultured shellfish through feeding a bacterial diet to the shellfish. To this aim, bacteria demonstrating toxin degradation will be micro-encapsulated to prevent primary digestion and to allow the delivery of a concentrated pulse of bacteria to the shellfish hepatopancreas. Shellfish feeding trials commenced to determine the uptake kinetics of encapsulated beads as a 'proxy' for the toxin degrading bacteria (Fig. 6). Trials were useful to show not only do both mussels and scallops ingest alginate microcapsules, but that the alginate is dissolved to release the indigestible beads - so for alginate capsules containing the bacteria they would be released in the digestive organs where they could potentially degrade any toxins.

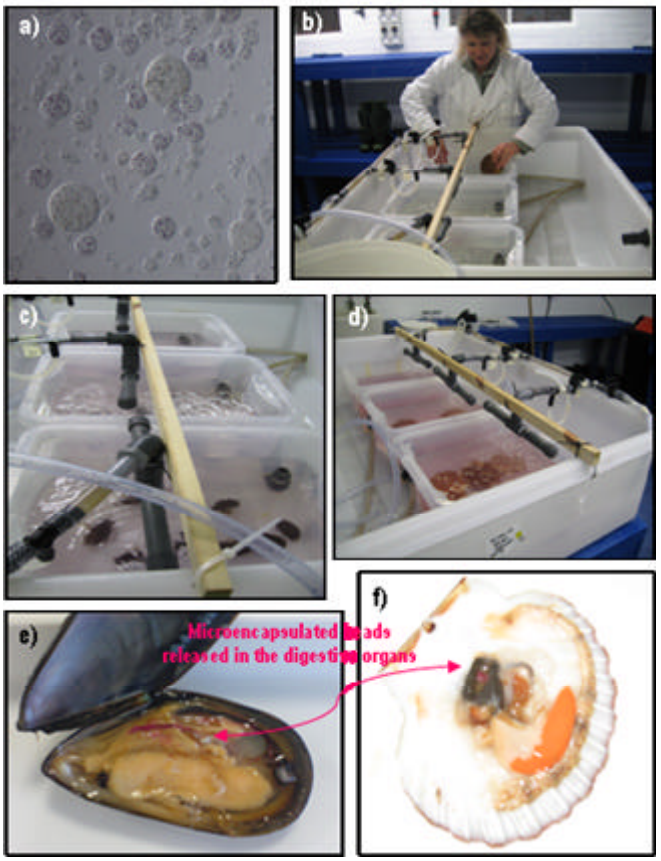

Figure 6. a) Microencapsulated beads, as a proxy for toxin degrading bacteria, (yellow 100-250 µm; red 50-100 µm, blue 0-50 µm) were b) fed to c) mussels and d) scallops. e & f) Both mussels and scallops ingested capsules over 2 hours period. Smaller capsules (pink beads) were more readily ingested by both mussels and scallops

Work-package 6: To set up new industry protocols designed to use latest Quality Control technology and set up a training regime, which can be used to bring the necessary skills to industrial users and to educate new researchers.

Training objectives of the SPIES-DETOX project are strongly interrelated to establishing adequate HACCP systems for marine biotoxins, both in terms of early warning measures, in-process controls/own-checks, and 'on-site' end-product testing. In order to identify the training needs and to design the training, meetings with major industry partners were held in Scotland and Spain. HACCP industry case studies were performed, and these studies will form the basis for the proposal of a best practice procedure for algal toxins. The concept of a best practice HACCP model will be important to give the training relevance and credibility.

To date, training courses on the use of ASP, PSP and DSP toxin detection kits (Fig. 7), suitable for use in commercial processing facilities and integration into HACCP plans, were provided in Scotland, Spain and Norway for participants from the shellfish industry.

Figure 7. Training aquaculturists in the use of simple, sensitive and robust assays for the detection of algal toxins

Subsequently, two demonstration sites were established at operations in Scotland and Spain. The operators were provided with rapid test kits for evaluation and their potential implementation for routine use. The dialogue and feedback with the demonstration sites provides useful information on where efforts should be focused on future rapid assay training and HACCP implementation by shellfish processors.

A further SPIES-DETOX workshop 'Integrating new marine biotoxin management tools in HACCP and food safety management' is now planned in Galway, Ireland in September 2009. The workshop is aimed at shellfish industry stakeholders, such as technical or lab managers, analysts, food safety and quality control/assurance personnel wishing to gain insight into the available front line tools in food safety management. Further information can be obtained from Hans Kleivdal (hans.kleivdal@biosense.com) or Majella Maher (Majella.Maher@nuigalway.ie).

Conclusion

To date, all partners involved in SPIES-DETOX have benefited from the focused research and field studies in the work-packages. It is envisaged that results from SPIES-DETOX will assist in the management of commercial shellfish aquaculture and help the industry pursue a successful future.

References

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Tillman et al. (2009) Eur J Phycol 44:63-79
Stewart et al. (1998) Can J microbiol 44:456-464
Smith et al. (2001) App Env Microbiol 67:2345-2353
Donovan et al. (2008) Toxicon 52:91-100

APPENDIX 1 – List of attendees and speakers

Surname	Forename	Company/Institute
Anrein	Rudi	SFPA
Butter	Tim	En-Force Laboratories Ltd.
Butler	Catherine	BIM
Chute	Killian	SFPA
Ciubotaru	Dan	BIM
Corish	Cormac	SFPA
Creed	Karen	EPA
De Burca	Stiofan	SFPA
Dullea	Michael	SFPA
Ellard	Ray	FSAI
Falvey	John	SFPA
Fitzgerald	Brian	SFPA
Flynn	Richie	IFA Aquaculture
Gallagher	Jerry	North West Shellfish
Gallagher	Dominic	SFPA
Gilmartin	Maeve	Marine Institute
Guilfoyle	Fergal	BIM
Hanley	Gerry	Bantry Bay Seafoods
Harrington	John	Kush Shellfish
Hatfield	Robert	CEFAS
Heffernan	Peter	Marine Institute
Hill	Pete	BIM
Hurley	Gearoid	Bantry Bay Seafoods
Keaveney	Sinead	Marine Institute
Long	Kathrina	BIM
Lynch	Gerard	SFPA
McGowan	Niamh	BIM
Millard	Dave	BIM
Minihan	Michelle	FSAI
Morrison	Catherine	BIM
Mullery	Alan	SFPA
Murray	Paul	SFPA
Newman	Kate	BIM
O'Callaghan	Daniel	SFPA
O'Carroll	Terrence	BIM
O'Cinneide	Micheal	EPA
O'Connor	Timothy	BIM
O'Shea	Cliona	SFPA
O'Sullivan	Aileen	SFPA
O'Sullivan	Finian	ISA
Owen	Kathy	CEFAS
Peyronnet	Arnaud	SFPA

Petersen	Andrew	En-Force
Rapkova	Monika	CEFAS
Richez	Fabrice	BIM
Steele	Susan	BIM
Stubbs	Ben	CEFAS
Tessars	Monica	BIM
Valh	Virginia	SFPA

Speakers and Chairs

Chamberlain	Tara	Marine Institute
Clarke	Dave	Marine Institute
Dore	Bill	Marine Institute
Duffy	Conor	Marine Institute
Evans	John	Marine Institute
Keady	Evelyn	NUIG
Lyons	David	FSAI
Maher	Majella	NUIG
Nolan	Brian	SFPA
Salas	Rafael	Marine Institute
Silke	Joe	Marine Institute
Turrell	Elizabeth	FRS Laboratories

MEFS Publications

1. *Assessment of Water Quality Data from Kilkieran Bay, Co. Galway*
   Evin McGovern, A. Rowe, B. McHugh, J. Costello, M. Bloxham, Conor Duffy, Eugene Nixon (2001)

2. *Trace metal and chlorinated hydrocarbon concentrations in shellfish from Irish waters, 1997-1999*
   Evin McGovern, A. Rowe, B. McHugh, J. Costello, M. Bloxham, Conor Duffy, Eugene Nixon (2001)

3. *The fate of oxytetracycline in the marine environment of a salmon cage farm*
   R. Coyne, P. Smith, Christopher Moriatrty (2001)

4. *Winter nutrient monitoring of the Western Irish Sea - 1990 to 2000*
   Evin McGovern, Eileen Monaghan, M. Bloxham, A. Rowe, Conor Duffy, A. Quinn, Brendan McHugh, T. McMahon, M. Smyth, M. Naughton, M. McManus, Eugene Nixon (2002)

5. *Monitoring of zebra mussels in the Shannon-Boyle navigation, other navigable regions and principal Irish lakes, 2000 & 2001*
   Dan Minchin, F. Lucy, M. Sullivan (2002)

6. *Monitoring of tributyl tin contamination in six marine inlets using biological indicators*
   Dan Minchin (2003)

7. *Trace metal and chlorinated hydrocarbon concentrations in shellfish from Irish waters, 2000*
   Denise Glynn, Linda Tyrrell, Brendan McHugh, A. Rowe, Jim Costello, Evin McGovern (2003)

8. *Trace metal and chlorinated hydrocarbon concentrations in various fish species, landed at selected Irish ports 1997-2000*
   Linda Tyrrell, Denise Glynn, A. Rowe, Brendan McHugh, Jim Costello, Conor Duffy, A. Quinn, M. Naughton, M. Bloxham, Eugene Nixon, Evin McGovern (2003)

9. *Environmental quality and carrying capacity for aquaculture in Mulroy Bay Co. Donegal*
   T. Telfor, K. Robinson (2003)

10. *Trace metal and chlorinated hydrocarbon concentrations in shellfish from Irish waters, 2001*
    Denise Glynn, Linda Tyrrell, Brendan McHugh, A. Rowe, Eileen Monaghan, Jim Costello, Evin McGovern (2003)

11. *The Irish coral task force and Atlantic coarl ecosystem study report on two deep-water coral conservation stakeholder workshops held in Galway in 2000 and 2002 A. Grehan, R. Long, B. Deegan, M. O'Cinneide (2003)*

12 *The occurrence and risk assessment of the pesticide toxaphene in fish from Irish waters. (2003).*
   Brendan McHugh, Denise Glynn, Eugene Nixon, Evin McGovern (2003)

13. *Trace Metal and Chlorinated Hydrocarbon Concentrations in Various Fish Species Landed at Selected Irish Ports, (2001)*
    Linda Tyrrell, Denise Glynn, Brendan McHugh, A. Rowe, Eileen Monaghan, Jim Costello, Evin McGovern (2003)

14. *An epidemiological investigation of the re-emergence of pancreas. Disease in Irish farmed Atlantic Salmon (Salmo Salar L.) in 2002 M. F. McLoughlin, E. Peeler, K. L. Foyle, H. D. Rodger, D. O'Ceallachain, F. Geoghegan (2003)*

15 *Salmon Mortalities at Inver Bay and Mc Swynes Bay Finfish Farms, County Donegal, Ireland during 2003*
Margot Cronin, Caroline Cusack, Fiona Geoghegan, Dave Jackson, Evin McGovern, T. McMahon, Francis O'Beirn, M. O'Cinneide & Joe Silke (2004)

16. *Trace Metal and Chlorinated Hydrocarbon Concentrations in Shellfish from Irish waters, 2002*
Denise Glynn, Linda Tyrrell, Brendan McHugh, Eileen Monaghan, Jim Costello, Evin McGovern (2004)

17. *Review of the potential mechanization of kelp harvesting in Ireland*
Astrid Werner Stefan Kraan (2004

18. *Trace metal and chlorinated hydrocarbon concentrations in Various Fish species landed at selected Irish Port, 2002*
Linda Tyrrell, Mary Twomey, Denise Glynn, Brendan McHugh, Eileen Joyce, Jim Costello, Evin McGovern (2004)

19. *Proceedings of the 5th Irish Shellfish Safety Scientific Workshop (2005)*

20. *Trace metal and chlorinated hydrocarbon concentrations in Various Fish Spiecies landed at selected port 2003*
Linda Tyrrell, Brendan McHugh, Denise Glynn, Mary Twomey, Eileen Joyce, Jim Costello, Evin McGovern (2005)

21. *Karenia mikimotoi: An exceptional Dinoflagellate bloom in western Irish waters, Summer 2005. J.Silke, F.O'Beirn and M. Cronin (2005)*

22. *Research on Pancreas Disease in Irish Farmed Salmon 2004/2005 - Current & Future Initiatives*
Neil Ruane, H. Rodger, D. Graham, L. Foyle, A. Norris, J. Ratcliff, K. Murphy, S. Mitchell, C. Staples, H. Jewhurst, D. Todd, Fiona Geoghegan, M. O'Cinneide (2005)

23. *Proceedings of the 6th Irish Shellfish Safety Workshop, Galway, 1st December 2005 (2006)*

24. *Guidelines for the assessment of dredge material for disposal in Irish waters.*
Margot Cronin, Evin McGovern, Terry McMahon, Rick Boelens, Marine Environment Consultant - Portroe, Nenagh, Co. Tipperary & Corresponding author (2006)

25. *Trace Metal Concentrations in Shellfish from Irish Waters, 2003.*
B. Boyle, Linda Tyrrell, Brendan McHugh, Eileen Joyce, Jim Costello, Denise Glynn, Evin McGovern (2006)

26. *Investigation into levels of dioxins, furans, polycholorinated biphenyls and brominated flame retardants in fishery produce in Ireland.*
ChristinaTlustos, Brendan McHugh, Iona Pratt, Linda Tyrrell, Evin McGovern (2006)

27. *Proceedings of the 7th Irish Shellfish Safety Workshop (2007)*

28. *Isolation and purification of AZAs from naturally contaminated materials, and evaluation of their toxicological effects*
Phillip Hess, Pearse McCarron, Nils Rehmann,Jane Kilcoyne, Terry McMahon, Gavin Ryan, Michael P. Ryan, Michael J. Twiner, Gregory J. Doucette, Masayuki Satake, Emiko Ito, Takeshi Yasumoto (2007)

29. *Bonamia Ostrea in the native oyster Ostrea edulis: A Review. Sarah Culloty and Marie Mulcahy (2007)*

30. *Infectious pancreatic Necrosis Virus and its Impact on the Irish Salmon Aquaculture and Wild Fish Sectors.*
Neil Ruane, Fiona Geoghegan, M. O'Cinneide (2007)

31. *Management recommendations for the sustainable exploitation of mussel seed in the Irish Sea*
J. A. Maguire, T. Knights, G. Burnell, T. Crowe, Francis O'Beirn, D. McGrath, M. Ferns, N. McDonough, N. McQuaid, B. O'Connor, R. Doyle, C. Newell, R. Seed, A. Smaal, T. O'Carroll, L. Watson, J. Dennis, M. O'Cinneide (2007)

32 *Issues and recommendations for the development and regulation of marine aggregate extraction in the Irish sea Cathal O'Mahony, Gerry Sutton, Terry McMahon, Micheal O'Cinneide, Eugene Nixon (2008)*

33. *Proceedings of the 8th Irish Shellfish Safety Workshop.*
Terry McMahon, B., Deegan, Joe Silke, Micheal O'Cinneide (2008)

34. *Pancreas disease in farmed salmon – health management and investigations at Irish farm sites. 2005 - 2008*

Neil Ruane, David Graham, Hamish Rodger (2008)

35. *Pilot water quality monitoring station in Dublin Bay north bank monitoring station (NBMS) MATSIS Project Part 1 Garvan O'Donnell, Eileen Joyce, Shane O'Boyle, Evin McGovern (2008)*

36. *Irish Sea Marine Aggregate Initiative (IMAGIN), Technical Synthesis Report Gerry Sutton. (2009)*

37. *Proceedings of the 9th Irish Shellfish Safety Workshop (2009)*