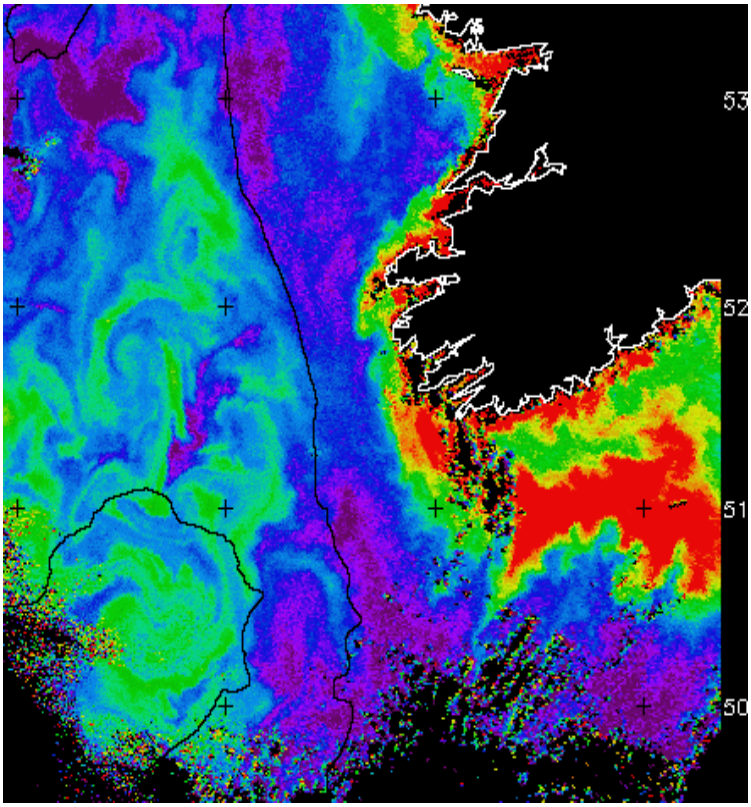
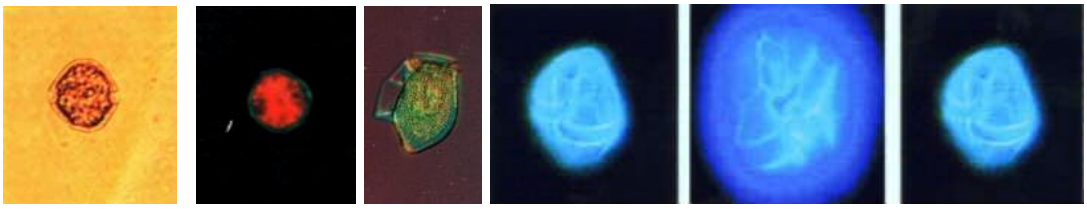
Marine Institute
Foras na Mara

Bord Iascaigh Mhara
Irish Sea Fisheries Board

Proceedings of the Second Irish Marine Biotoxin Science Workshop



Galway 11th October 2001



2nd Irish Marine Science Biotoxin Workshop

Galway, Thursday 11th October 2001

Organised by the Marine Institute and Bord Iascaigh Mhara with support of the Irish Shellfish Association

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OPENING SPEECH

Mr Hugh Byrne TD, Minister of State at the Department of the Marine and Natural Resources

Ladies and Gentlemen

I am delighted to be here to welcome you all to the 2nd Irish Marine Science Biotoxin Workshop. This is an important forum, because it is the only time in the year when we bring together all the key Irish players in the sector, from industry and science to the regulators – together to take stock of where we currently stand on the issue of biotoxins. Indeed I would like to particularly welcome our colleagues from Northern Ireland and the UK who have travelled to be in attendance today.

Biotoxins is the issue possibly uppermost in the minds of many in the Irish shellfish industry during the last two years. There is probably general agreement that the future growth prospects and overall health of the sector hinge crucially on the real-time effectiveness of biotoxin monitoring.

As you are no doubt aware, I have over the last two and half years dedicated a considerable amount of my time to this issue. This has resulted in my acquiring knowledge of the sector that was hitherto unknown for any Minister with responsibility for Aquaculture. I believe that major strides have been made since my visit to New Zealand last year with a group comprising Industry and Departmental representatives to see at first hand the developments taking place there.

Following our fact finding missions to New Zealand, we have agreed that the Irish aim should be to provide the best biotoxin monitoring system in the Northern hemisphere, on a par with that of New Zealand, where some 70,000 tonnes of mussels are successfully produced annually. Teamwork is also a key to their success.

The Irish biotoxin monitoring system has been revamped in the past year. The has involved significant capital investment and the recruitment of eight specialist staff at the Marine Institute – all aimed at reduced frustration within the industry and with satisfying requirements imposed by the EU and the Food Safety Authority of Ireland. You now receive weekly reports on phytoplankton levels, the chemical testing for AZP is underway and the amended bioassay step was phased in since April 2001. The national programme now costs over £1 million per year to run and administer.

Co-ordination and teamwork has been crucial to progress in the biotoxin issue. I welcome the close co-operation, which has resulted from my active involvement in this process. I refer of course to the industry, the Department and it's Agencies under the rubric of the Molluscan Shellfish Safety Committee. Indeed I would like to thank the Food Safety Authority, the Marine Institute, and the Shellfish managers of my own Department for their committed and effective response that they have faced in overcoming the difficulties in the sector.

Following the 12 regional meetings with the industry in November and December 2000, a number of key steps have been taken to improve the monitoring procedures and to facilitate the opening of productions areas wherever feasible.

I met with the EU Commissioner for Food Safety, David Byrne in April of this year and secured his endorsement for a harmonised testing regime throughout the whole Community, as well as the need to improve methods for testing shellfish in line with the latest available international technology. Specialists from the Food Safety Authority, the Marine Institute and leading Irish researchers subsequently took part in the Working Group on Toxicology of DSP and AZP in Brussels in May.

This is an issue I am pressing actively with the Commission and we ask that our European partners work closely with us in achieving common standards in this key area of food safety.

All in all, I welcome the working evidence of new monitoring systems. Today's meeting will give an opportunity to assess how the regime is progressing, what research is underway and what further steps must be taken to strengthen the work already completed. I would ask, no insist that we would have the full co-operation of producers and for the continued links with researchers in the Marine Institute and at other facilities, as we fine-tune and improve our systems.

The Marine Institute has commissioned Dr. Robin Raine, NUI Galway to produce a new guide to Irish Phytoplankton, which is an essential tool in the monitoring and research of algal events. I understand that the final draft has now been produced and would like to thank Robin and his colleagues for their efforts to date. This guide will be going to press shortly and I look forward to seeing it in circulation throughout the Sector.

Finally, I welcome the international dimension in today's programme and in particular we look forward to hearing from Lincoln McKenzie and Don Anderson. We hope that international research involving the Marine Institute and partners such as NOAA in the United States will result in a deeper understanding of the factors that cause algal blooms and toxicity.

In conclusion I would re-iterate to everyone here today that my goal has been to oversee the introduction of a biotoxin early warning system, specifically designed for Irish conditions and this remains my commitment to the Sector.

I can assure you of my continued support and involvement and I wish your workshop every success.

OBJECTIVES AND SCOPE OF THE MARINE BIOTOXIN SCIENCE WORKSHOP

Michéal Ó Cinnéide, Marine Institute, Marine Environmental & Health Services Division

The Marine Institute's objectives for Irish Biotoxin programme are to support the continued development of the Irish Shellfish Industry and to promote food safety, by building the best Biotoxin Management System in the Northern Hemisphere.

The Marine Science Biotoxin Workshops are part of Marine Institute's role as the National Reference Laboratory. The EU mandate for Reference Labs emphasises the need for dissemination of information.

1. Objectives of the Marine Science Biotoxin Workshop

- Take Stock of developments since last Workshop, April 2000
- Review Irish Monitoring System & Trends
- Summarise current Irish Research in HAE/ Phytoplankton
- Compare with International Best Practice in New Zealand and USA
- Provide a Forum for Debate/ Feedback

2. Models

There are several international models for this type of Workshop.

- New Zealand Workshops, held twice annually by MAF since 1994
- Australia - "Research Network for Algal Toxins"
- USA - "Symposium on Harmful Marine Algae", December 2000

3. Key Irish Developments since April 2000

The Irish aquaculture industry had sought increased resources for biotoxin monitoring since the Biotoxin Taskforce in 1995. There have been major changes in the Irish biotoxin-monitoring regime since the previous workshop was held in Cork in April 2000. These include:

- Restructuring of Molluscan Shellfish Safety Committee, Sept. 2000
- 13 Regional MSSC meetings with industry, November – December 2000
- Change in the bioassay method to DEE extraction, April 2001
- Phytoplankton - weekly monitoring and results since January 2001
- Chemistry - weekly testing for ASP, DSP & AZP, May 2001

These changes have come as a result of intensive discussions between the agencies and the Irish shellfish industry. Minister of State Hugh Byrne TD played a key role in bringing a high level focus on the issues.

4. Irish Research in Harmful Algal Events / Biotoxins

The incidence of biotoxins in Irish coastal waters had brought serious hardship for Irish aquaculture producers. In order to address this problem, the Marine Institute and Irish third level institutions have got involved in a range of research initiatives. The current status on several of these projects will be reviewed at the workshop:

- Biotoxin Unit, Marine Institute carries out survey and research cruises on dinoflagellate cysts, plankton, and oceanography.
- Marine Institute does collaborative research with staff from the Martin Ryan Institute for Marine Science, NUI Galway on phytoplankton and oceanography.
- Marine Institute has funded work at Bio Research Ireland, Galway on the development of new cell assays and immuno assays to detect biotoxins
- Cork Institute of Technology and UCC are doing a HEA funded project on ASP

5. Marine Institute International Best Practice / Research

As the impact of biotoxins on shellfish and human health is a global phenomenon, the Marine Institute has sought to build international links, with a view to bringing the best expertise to bear on the problems.

- Marine Institute Biotoxin Unit is a partner with the Marine Lab, Aberdeen and with the Department of Agriculture & Rural Development, Northern Ireland, Belfast on isolation of AZP standards

- Marine Institute has developed links with NOAA (USA) and with the Woods Hole Oceanographic Institution (WHOI) in Harmful Algal Events research

- Marine Institute staff had a training visit to Tohoku University, Japan on techniques for AZP isolation and purification in mid 2001

- The Marine Institute co-operates with the Cawthron Institute, New Zealand, which has a leading role in the monitoring of biotoxins for the New Zealand shellfish industry e.g. exchange visits, development of gene probes for phytoplankton and new techniques for chemical testing.

- The Marine Insitute has close links with the EU Biotoxin Reference Lab, Vigo in the area of inter-calibration and research methods. The Institute works with the EU Commission, DG Sanco and the Food & Veterinary Office (FVO) - for example Marine Institute personnel have taken part in FVO missions to Thailand, Sweden and the Netherlands in 2000-2001.

AN OVERVIEW OF THE BIOTOXIN MONITORING PROGRAMME IN 2001

Terry McMahon, Joe Silke, Philipp Hess, Dave Clarke, Leon Devilly, Deirdre Slattery, David Swords, Geraldine Dowling, Maria McCarron, Billy Gibbons, Fearga Walsh, Tara Chamberlain, Caroline Cusack and Michéal Ó'Cinnéide.
Marine Institute, Snugboro Road, Abbotstown, Dublin 15.

Introduction

The national marine biotoxin monitoring programme is co-ordinated by the Marine Institute's Biotoxin Unit based in Abbotstown in Dublin. The programme involves the routine testing of shellfish samples for the presence of DSP, AZP, PSP and ASP toxins as well as the microscopic analysis of water samples for the identification and quantification of toxin producing algal species. The results of the analysis are issued on regular basis to the Food Safety Authority, Department of the Marine and Natural Resources, shellfish producers and shellfish processors by fax and SMS text messages via mobile phone.

Numbers of samples tested

The numbers of samples tested during 2000 and during the period January - September 2001 are given in Table 1. There was a significant increase in the numbers of samples tested in 2001 compared to 2000. The number of DSP bioassays carried out increased from 3129 during 2000 to 3242 during the period January to September 2001 and it is estimated that the total number of DSP bioassays that will be carried out during 2001 will exceed 4000. In the case of PSP bioassays the numbers increased from 178 during 2000 to 306 in 2001. The large increase in the number of PSP assays carried out was partly due to the testing of shellfish from production areas along the west coast following the detection of high levels of the PSP toxin producing algae *Alexandrium tamarense* in these areas in July / August of 2001. In 2000 no chemical analysis of DSP and AZP toxins was carried out but in 2001, with the recruitment of additional staff and the purchase and installation of an LC-MS system, the routine chemical analysis of these toxins was introduced. Details of DSP and AZP analysis of samples by LC-MS are given in the paper by Hess *et al* elsewhere in these proceedings. The number of phytoplankton samples analysed also increased significantly and additional details of the phytoplankton monitoring programme can be found in the papers by Caroline Cusack and Tara Chamberlain in these proceedings.

Table 1. Number of samples tested during the national monitoring programme in 2000 and from January - September 2001.

Analysis	2000	2001 (January -September)
DSP bioassay	3129	3242
PSP bioassay	178	306
ASP - HPLC analysis	738	500
DSP/AZP - LC-MS analysis	0	1286
Phytoplankton analysis	1231	1633

Mouse Bioassay

During 2000 the Yasumoto 1978 mouse bioassay protocol was used for testing of samples for the presence of DSP toxins. In April 2001 following discussions between the Food Safety Authority of Ireland, the Department of the Marine and Natural Resources representatives of the shellfish industry and the Marine Institute, a revised protocol, (Yasumoto 1984) was introduced. The revised protocol was introduced to minimise the potential interference of low levels of Yessotoxins with the assay result. The Yasumoto 1984 bioassay involves the following steps.

- Dissect out 25g of hepatopancreas
- Extract the hepatopancreas 3 times with acetone
- Evaporate off the acetone in a rotary evaporator
- Extract the residue 3 times with diethylether
- Evaporate off the diethylether in a rotary evaporator
- Dissolve the residue in 1% Tween 60
- Inject 1ml into each of 3 mice (19-21g)

The total preparation time for 1 sample is approximately 2.5 hours. A positive result is indicated if 2, or more, of the 3 mice injected are dead within 24 hours.

Of the 3242 DSP mouse bioassays carried out during the period January to September 2001 a total of 1938 were carried out using the Yasumoto 1978 protocol while the remaining 1204 were carried out using the Yasumoto 1984 protocol. Some 17.6% of the samples tested using the Yasumoto 1978 protocol gave positive results while 16.6% of the samples tested using the Yasumoto 1984 protocol gave positive results.

In addition to routine testing, the Marine Institute also organised an inter-comparison study between the 3 laboratories, Marine Institute Dublin, BESU Cork and BLE Ballina, involved in the testing of samples by mouse bioassay. There was excellent agreement between the results obtained in each laboratory indicating that the results obtained in each laboratory are directly comparable. Each of the laboratories is also actively in the process of seeking ILAB accreditation of the method thus ensuring that the highest standards of sample testing procedures are achieved and maintained.

Sample Codes

The Marine Institute, in co-operation with the Department of the Marine and Natural Resources and BIM, have been involved in defining and mapping production areas and defining, mapping and assigning unique codes to sample locations. The codes will be used throughout the system, from sample collection to the issuing of results, and will ensure that the samples can be unambiguously traced back to the location from which they were taken.

Reporting of results

The timely processing and reporting of results of all tests is a key element in the biotoxin monitoring programme. Currently all results are compiled in the Marine Institute and reports prepared and issued by FAX. During the period January to September 2001 a total of 234 individual reports were issued compared with a total of 194 during all of 2000. SMS text messaging via mobile phone was introduced in 2001 to increase to the speed with which producers and processors are advised of test results.

A total of 84% of all DSP test results were issued with 72 hours of the sample being taken and efforts are being made to reduce the lag time being samples being taken and the results of the laboratory analysis being issued. The Marine Institute's Marine Data Centre and Biotoxin Unit are developing a Web based information system that will make test results and relevant information more readily accessible. It is hoped to have the new system in place in 2002.

Acknowledgements

The Marine Institute would like to acknowledge the help, co-operation and hard work of all involved in the monitoring programme including the staff of BESU and BLE, the Sea Fisheries Officers of the Department of the Marine and Natural Resources, BIM, FSAI and the industry.

BIOTOXIN CHEMICAL MONITORING IN IRELAND-2001

Philipp Hess, Terry McMahon, Deirdre Slattery, David Swords, Geraldine Dowling, Maria McCarron, David Clarke, Leon Devilly, William Gibbons, Joe Silke, Michéal O'Cinnéide. Marine Institute, Snugboro Road, Abbotstown Laboratory Complex, Dublin 15, Ireland.

Introduction of chemical testing

During 2001, the Marine Institute has extended its range of chemical tests to include analysis by High Performance Liquid Chromatography with Ultraviolet Detection (HPLC-UV) and Liquid Chromatography coupled to Mass Spectrometry (LC-MS). Thus the range of compounds determined extends from domoic acid to DSP compounds, such as okadaic acid (OA) and dinophysis toxins (DTXs) to azaspiracids (AZA-1 to -3). These tests complement the mouse bioassay, which is the current reference method within the European Community. The chemical tests serve mainly to detect compounds that cannot be detected by the mouse bioassay at levels dangerous to human health (domoic acid and azaspiracids), and the identification of compounds also helps to explain the cause of toxicity present (okadaic acid and DTXs). A total of 1800 shellfish tissues have been analysed from January to September 2001.

Multi-toxin Method

Okadaic acid, DTXs and azaspiracids are routinely monitored in shellfish flesh from all producing areas in Ireland, in parallel to the mouse bioassay. An extraction is carried out from a single homogenate by blending a 4 g aliquot with 15 ml of 80 % aqueous methanol. The crude extract is then centrifuged and filtered using a 0.2 µm syringe filter, prior to injection into the LC-MS. All compounds are analysed using a method with a single run by LC-MS-MS, often referred to as a multi-toxin method. The mass spectrometer used is a triple-stage quadrupole allowing tandem mass spectrometric analysis, which is considered sufficiently confirmatory to provide evidence in court in cases of illegal drugs or compounds used above the legal limits. The tandem mass spectrometry gives additional information on the compound analysed as shown in Figure 1. In this method okadaic acid elutes first at ca. 7 min, followed by DTX-2 at 7.5 min and the azaspiracids, eluting at approximately 10 – 12 min (Figure 2). Quantification is carried out using multi-point calibration curves made from certified standards or, where these standards are not available, from standards supplied by internationally accepted authorities, i.e. Profs. Yasumoto and Satake. Quality control checks are carried out to internationally accepted protocols, and include analysis of certified reference materials whenever available. The total run time per sample on the LC-MS has been optimised to 15 mins. This allows a fast sample turnaround of ca. 2-3 days from receipt of a shellfish sample in the laboratory.

DSP and AZP in mussels, oysters and razor fish

The level of okadaic acid (OA) equivalents in shellfish was set by comparison with the mouse bioassay to 0.16 µg/g (draft EU recommendation 2227). Mussels (*Mytilus edulis*) were the species most affected by the occurrence of DSP toxins, with an average level of 0.99 µg/g for the 452 samples analysed (Table 1). The maximum level observed was 48.3 µg/g, and the limit of 0.16 µg/g was exceeded in 36 % of the cases examined. This percentage is higher than the total percentage of production areas affected since the chemical analysis was initially only concentrated on the problem areas where positive mouse bioassays were observed. *Crassostrea. gigas* were much less affected with an average level of 0.06 µg/g and a maximum concentration of 7.8 µg/g. The limit was only exceeded in 6 (2%) of the 306 *C. gigas* examined. None of the *Ostrea edulis* or the *Ensis siliqua* showed concentrations above the limit of 0.16 µg/g.
The Irish Food Safety Authority set the limit for azaspiracids in shellfish to 0.16 µg/g, following risk assessment. This limit was exceeded in *M. edulis* only in 8.5 % of all cases examined (452 samples, May to October 2001). Oysters were even less affected with only 1.6 % of all *C. gigas* samples exceeding the limit (306 samples, May to October 2001). None of the *Ostrea edulis* (31 samples) or *E. siliqua* (26 samples) exhibited concentrations above the limit.

Table 1 Okadaic Acid and azaspiracid equivalents in mussels, oysters and razor fish

OA equivalents in mussels, oysters and razor fish

C [µg/g]	Mean	Maximum	% > 0.16 µg/g
Mytilus edulis (452)	0.99	48.3	36 %
Crassostrea gigas (306)	0.06	7.76	2 %
Ostrea edulis (31)	<0.01	0.05	0 %
Ensis siliqua (26)	<0.01	0.02	0 %

AZP - equivalents in mussels, oysters and razor fish

C [µg/g]	Mean	Maximum	% > 0.16
Mytilus edulis (452)	0.07	1.5	8.5 %
Crassostrea gigas (306)	0.03	0.42	1.6 %
Ostrea edulis (31)	< 0.01	0.04	0 %
Ensis siliqua (26)	< 0.01	0.08	0 %

Comparison of LC-MS with mouse bioassay

When comparing the results from the LC-MS method with those from the mouse bioassay, a large concordance was found over a three-month test period (415 shellfish samples), Figure 3. Approximately 80 % of the samples

were negative using the two techniques and 13 % were positive using both techniques. These results therefore give a 93 % agreement between techniques. The discrepancies in the remaining 7 % were 5 % of mouse bioassay negatives with positive chemical analysis, and only 2 % of chemical negatives with positive mouse bioassays. The mouse bioassay negatives with a positive chemical result are likely to be explained by the error margin of either test (ca. 10 – 20 %). The negative chemical results with a positive mouse bioassay may be a sign for the presence of other toxins, which were not analysed for by LC-MS. This comparison will be extended to cover a longer period. The results will then be presented to the European National Reference Laboratories for the evaluation of chemical testing as a method alternative to the mouse bioassay.

Scallop toxicity

King scallops (*Pecten maximus*) have posed a problem in several European countries (UK, Spain and Ireland), as well as internationally (Japan, US), in the monitoring of a range of marine biotoxins including domoic acid, saxitoxins and yessotoxins.

In Ireland, scallops have been monitored for domoic acid, the amnesic shellfish poisoning (ASP) toxin (since 1999), and diarrhetic shellfish poisoning (DSP) toxins (since August 2001). The King Scallop is one of the few bivalves that allow dissection of internal organs prior to sale on a commercially viable scale. The distribution of toxins between different organs of the scallop has been the subject of a number of international studies, all of which have shown that the hepatopancreas typically contains the highest concentrations and can account for over 90 % of the total amount of toxin present in each animal.

Around 500 scallop tissues have been analysed for the presence of domoic acid by the Marine Institute during the period from January to September 2001 (Table 2). The average content of domoic acid in white meat (adductor muscle) was 1.5 µg/g, and the maximum in this tissue only exceeded the limit on 2 occasions. The roe (gonads) showed higher values, with an average of 5.6 µg/g, a maximum of 69 µg/g and 8.1 % of all samples exceeding the limit of 20 µg/g. The hepatopancreas (HP) showed the highest concentrations with an average of 433 µg/g, a maximum of 2246 µg/g and 86.3 % of all the samples exceeding the limit of 20 µg/g. The remaining soft tissues (mantle, gills etc.) were intermediate in concentration and had an average content of domoic acid of 50 µg/g (11 % exceeding the limit). This distribution had been previously communicated to the Food Safety Authority of Ireland who recommends processing to all scallop producers in Ireland.

Since August 2001, scallops have also been screened for the presence of diarrhetic shellfish toxins (okadaic acid and DTXs) and azaspiracids. The 25 samples (100 tissues) examined so far showed little or no okadaic acid or azaspiracid equivalents in the adductor muscle, the roe or soft tissues other than the hepatopancreas. The hepatopancreas also showed relatively low levels, both compared to other species and compared to other toxins found in scallops. The maximum of OA equivalents found in scallop HP was 1.86 µg/g. Since the limit of 0.16 µg/g of OA equivalents was exceeded in 40 % of the HPs examined, dissection of the scallop would also be recommended from

the occurrence of DSP toxins alone. The distribution of azaspiracids was very similar to the one of DSP toxins, however, the maximum observed level was only 0.32 µg/g in the HP.

Table 2
Domoic acid in scallops, statistical summary

C [µg/g]	Mean	Maximum	% > 20
Meat	1.5	28.4	1.1%
Gonad	5.6	69.2	8.1 %
Hepatopancreas	433	2246	86.3 %
Remainder	50	1000	11.4 %
Whole flesh	56.1	370	38.6 %

OA - equivalents in scallops, statistical summary

C [µg/g]	Mean	Max	% > 0.16
Meat	0	0	0%
Gonad	0	0.03	0%
Hepatopancreas	0.23	1.86	40%
Remainder	0	0	0%
Whole	0.02	0.19	4 %

AZP - equivalents in scallops, statistical summary

C [µg/g]	Mean	Max	% > 0.16
Meat	0	0	0%
Gonad	0	0.02	0%
Hepatopancreas	0.1	0.32	28%
Remainder	0	0.01	0%
Whole	0.01	0.03	0 %

Case studies in Killary and Castletownbere

Killary Harbour and Bantry Bay are two areas that had been affected by high toxin levels for long time periods (over 10 months in 1995/6 and 2000/1). Three locations from within Killary Harbour were monitored at frequent intervals during this summer. The results show that inner Killary was less affected by toxicity than the other two regions, at least in terms of concentrations found by chemical analysis. Thus, mussels from the inner part of Killary never had AZA concentrations above the 0.16 µg/g, and only 4 weeks of levels over 0.16 µg/g of okadaic acid equivalents (Figure 4). The middle and outer part had both 9 weeks of the limits being exceeded for OA equivalents and 3 and 4 weeks, respectively, of the limits being exceeded for azaspiracids (Figure 4). Thus, we propose to further examine the oceanographic and environmental factors leading to such differences, and to examine the possibility to manage Killary Harbour as two different production areas. Castletownbere, in Bantry Bay, is presented as a case since the toxicity was relatively high during 2001 and since several separate toxic

events have occurred in this area. These events were distinguished by the occurrence of different phytoplankton species (*Dinophysis acuminata and D. acuta*) and by the occurrence of two different toxins, namely OA and DTX-2, a stereo-isomer of OA. Due to high levels in the hepatopancreas and whole flesh of mussels, the area was closed for shellfish harvest during the time reported (Figure 5). However, regular samples were obtained for chemical testing and mouse bioassays. The occurrence of okadaic acid dominated the pattern from May to June, while DTX-2 started to occur from July and peaked in August 2001, with 350 µg/g found in mussel hepatopancreas (Figure 6).

Summary

Overall, chemical testing has proven an invaluable tool in the assessment of shellfish toxicity in Ireland. The levels of azaspiracids were monitored for the first time routinely in shellfish samples since May 2001. The chemical testing has also shown good agreement with the biological assay (93 %) over a 3-month period. The LC-MS analysis has revealed that the toxicity during this year was mainly due to known DSP toxins, namely okadaic acid and DTX-2. The chemical testing also addressed issues of toxin distribution in scallops, and showed in accordance with other international studies, that processing of scallops is necessary to remove the highly toxic hepatopancreas.

We anticipate that the use of chemical techniques will increase to play a major role in monitoring and will help in the investigation of the origin, management and mitigation of algal toxins.

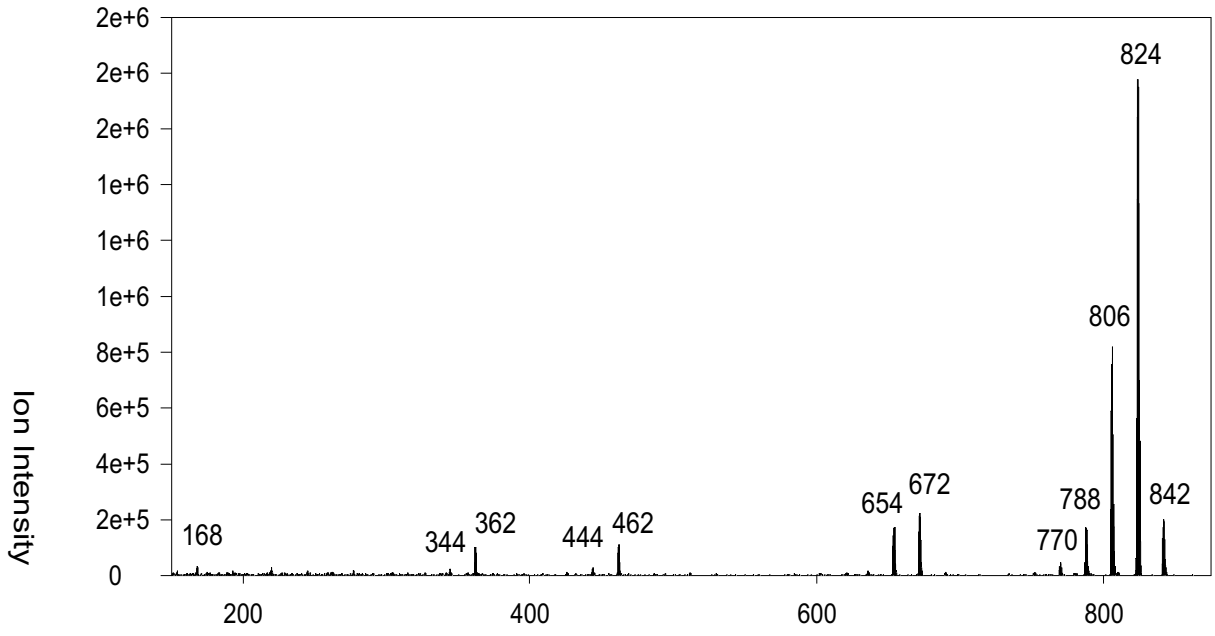

Figure 1. Example of mass spectrum obtained by LC-MS-MS, azaspiracid-1.

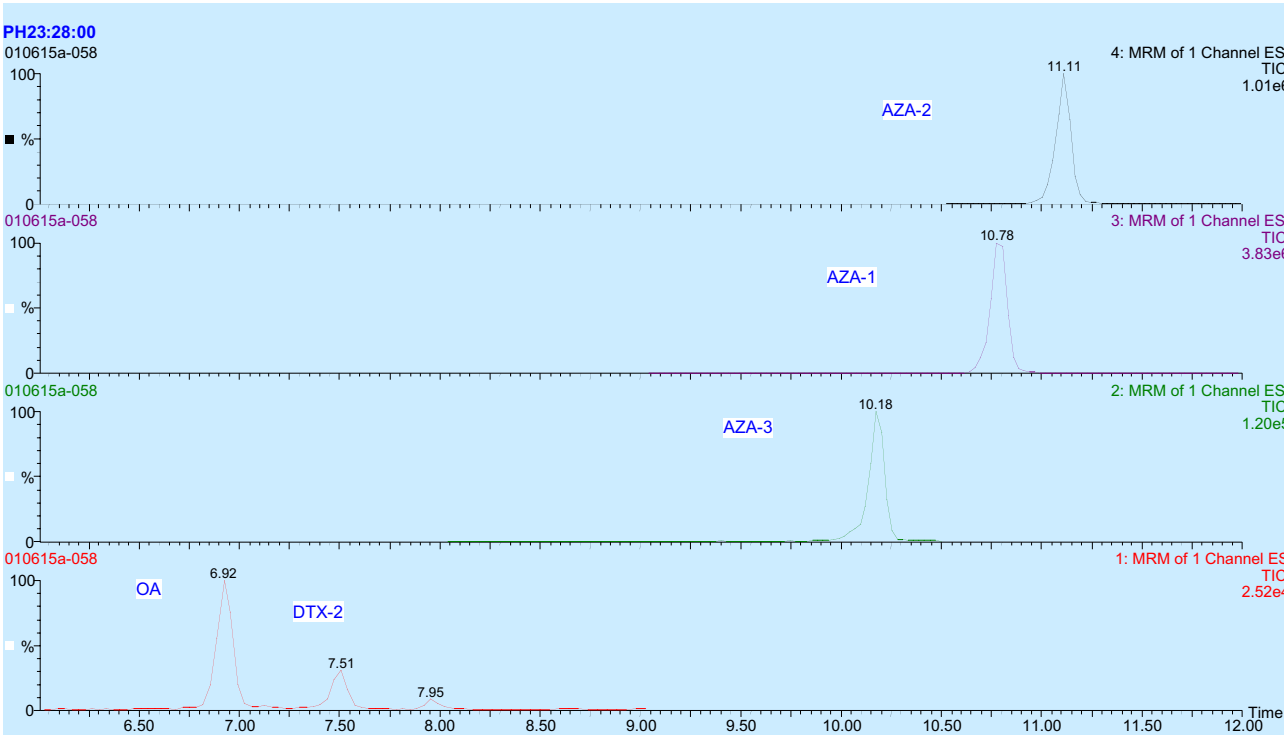

Figure 2. Multi-toxin method by LC-MS-MS, ion chromatograms

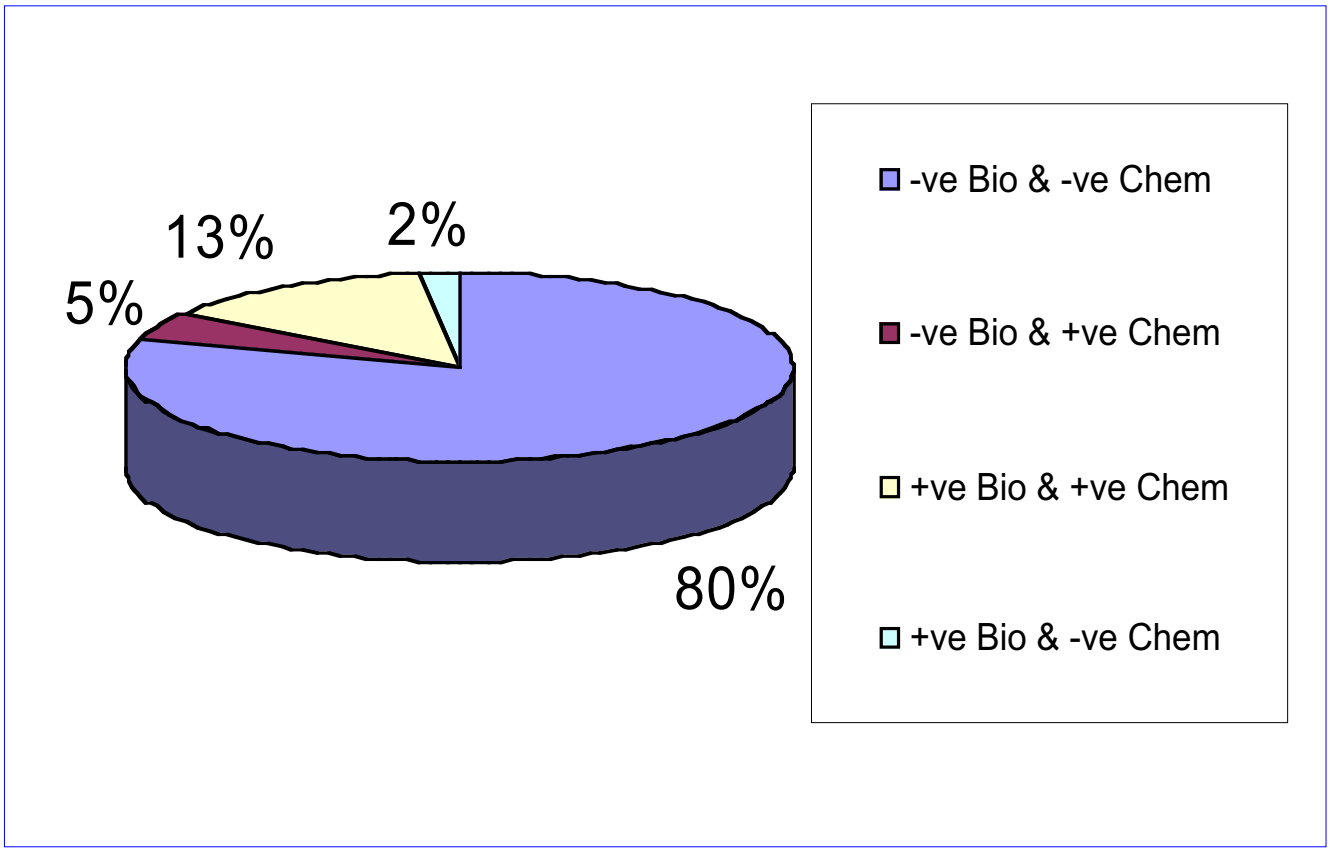

Figure 3. Comparison of results obtained by LC-MS-MS and the mouse bioassay.

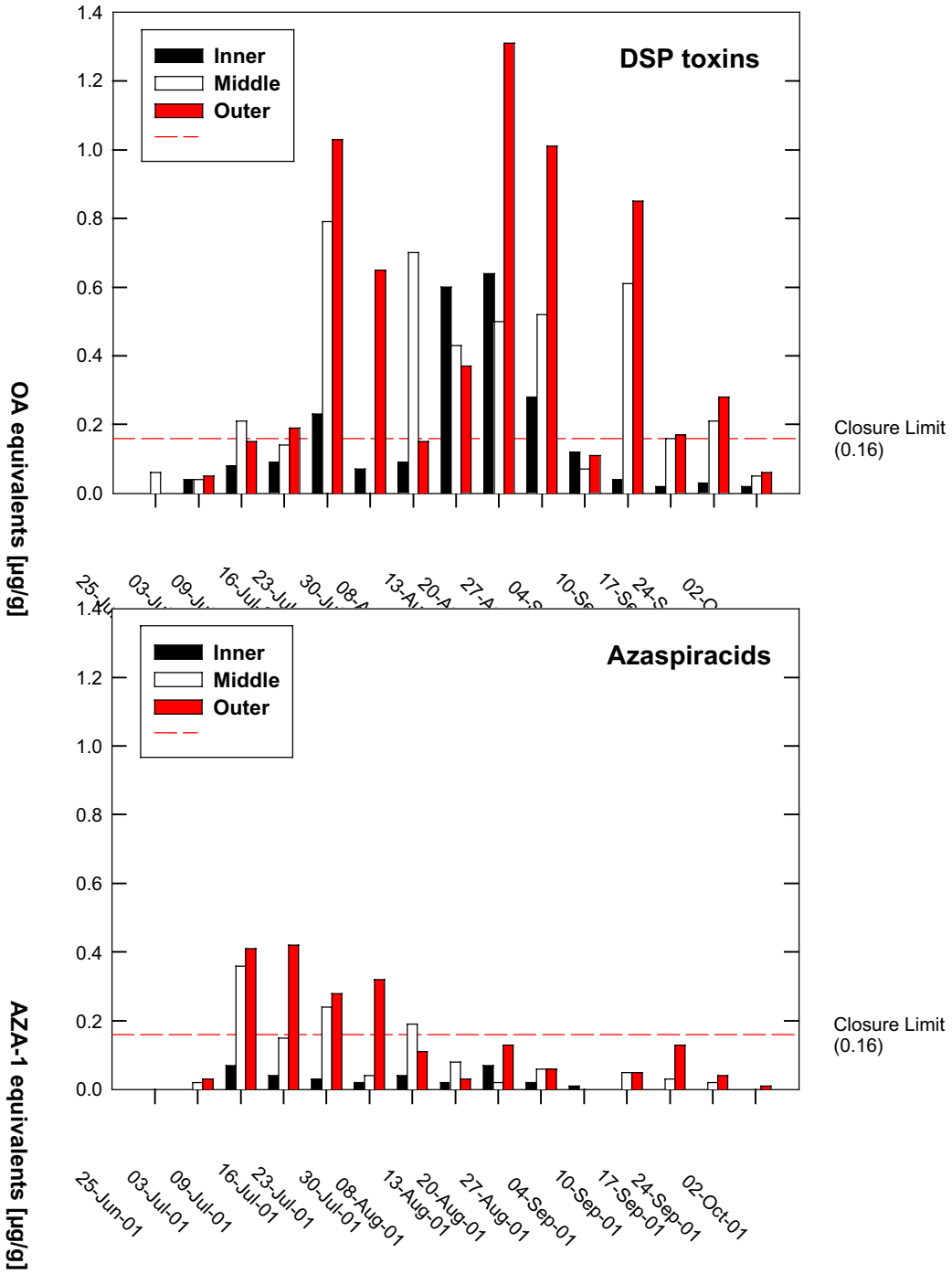

Figure 4. Okadaic acid (top graph) and Azaspiracid (bottom graph) levels in whole mussels from three regions within Killary Harbour, June to October 2001.

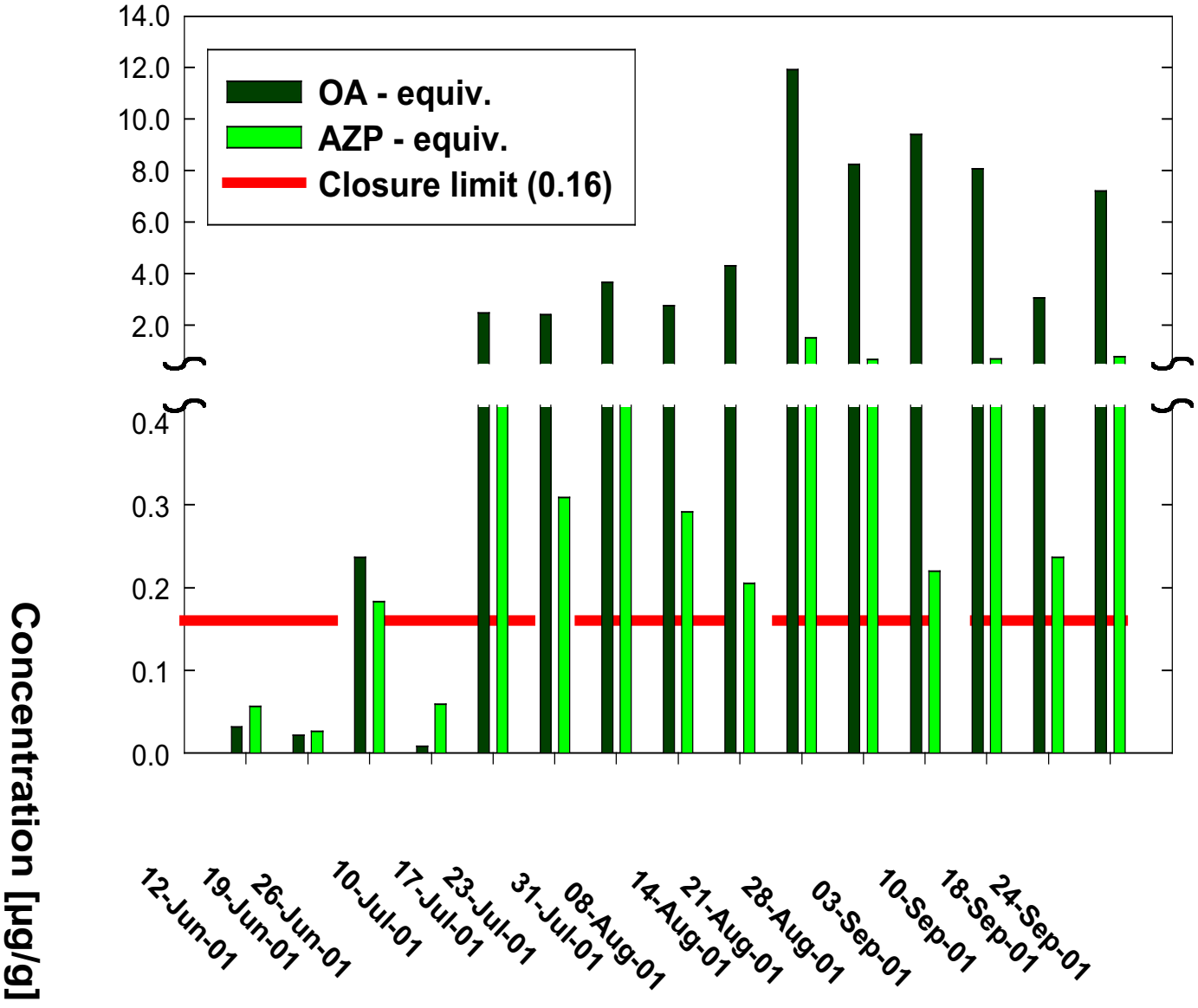

Figure 5. Okadaic acid and AZA-1 equivalents in whole mussels from Castletownbere, Bantry Bay.

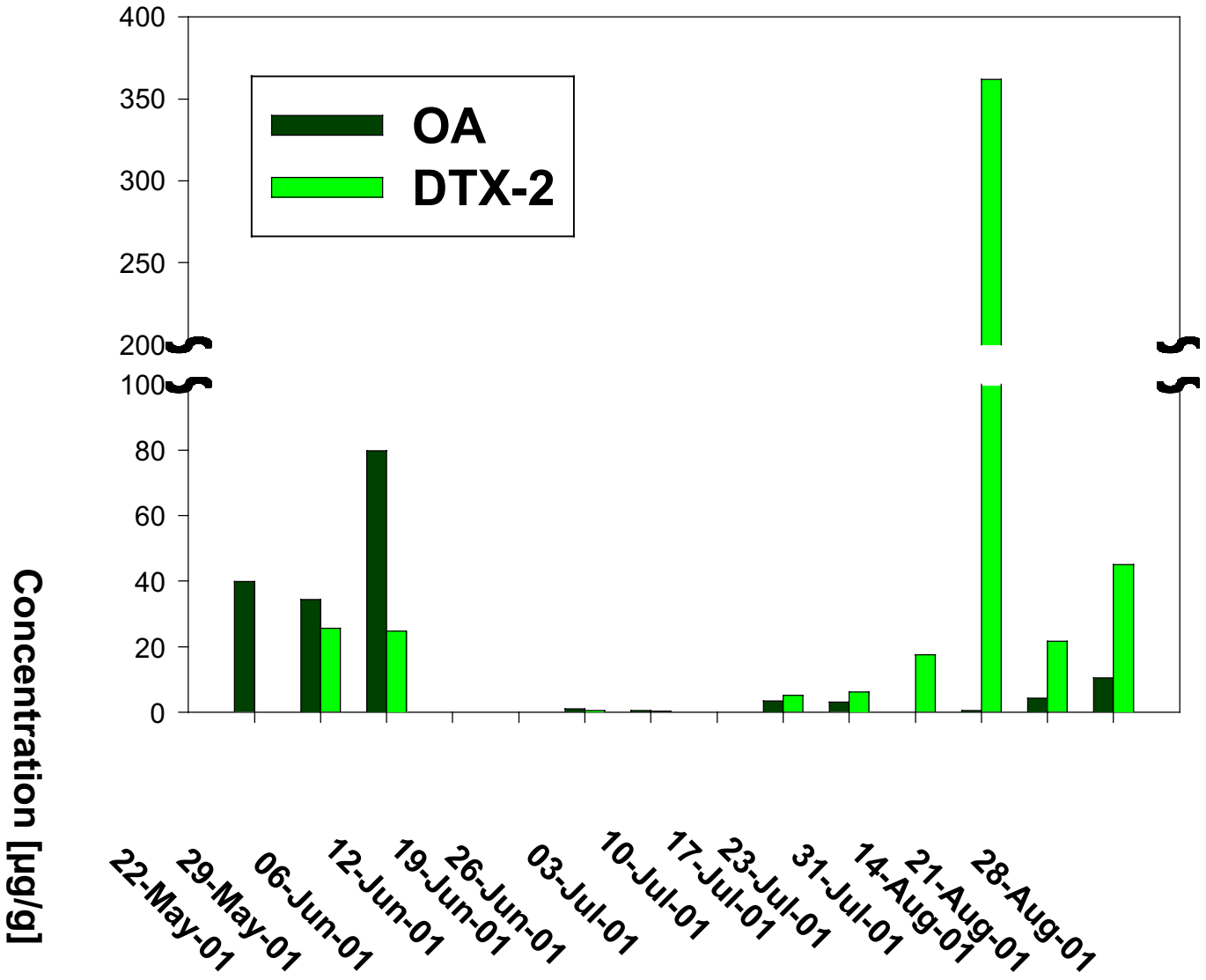

Figure 6. Okadaic acid and Dinophysistoxin-2 in mussel hepatopancreas from Castletownbere, Bantry Bay

SUMMARY OF PHYTOPLANKTON MONITORING TRENDS IN 2001

Caroline Cusack, Tara Chamberlain, Leon Devilly, Dave Clarke and Joe Silke.
Marine Institute

The purpose of this presentation was to provide information on the progress of the phytoplankton-monitoring programme in Ireland for 2001. Following discussions with the industry (regulators and growers) last year the monitoring programme has been modified. Two extra personnel were employed to cope with the increased number of production areas now monitored for phytoplankton.

The Marine Institute phytoplankton team carry out weekly analysis of water samples taken at approximately 60 sites from various aquaculture production areas countrywide. Already this year, 1,633 samples have been analysed (January to September 2001). The resulting data is transferred to the Marine Institute's Harmful Algal Event (HAE) database and weekly reports are sent out to the industry. One of the main objectives of this programme is to determine if relationships exist between the abundance and composition of toxic phytoplankton and the resulting toxicity levels in shellfish. Future attempts to model HAE's will require this type of data. Phytoplankton also often provides information regarding the nature of a toxic event or fish kills.

Phytoplankton composition

Off the west and north-west coasts during 2001, phytoplankton abundance increased towards spring. As expected the spring bloom consisted primarily of diatoms such as *Thalassiosira* sp., *Chaetoceros* sp., *Skeletonema costatum*, *Leptocylindrus danicu*s. The bloom was shortly followed by an increase in microflagellates. On the 26th April *in situ* underwater cameras at Killary Salmon Farms showed very poor visibility and phytoplankton samples contained relatively high cell densities of the Haptophyte *Phaeocystis* cf. *pouchetii*. Maximum cell numbers were recorded from Killary Harbour on May 14th with up to 7 million cells/L^{-1}. During the summer period, diatoms and dinoflagellates dominated the species spectrum. While the number of diatom species decreased slowly, an increase in dinoflagellate species was observed. The dinoflagellate *Alexandrium* cf. *minutum* was observed in samples examined at the end of July, and increased in cell concentrations during August. As some species within this genus can cause potentially fatal Paralytic Shellfish Poisoning (PSP) in humans, shellfish tissue samples from all production areas where this organism was present were tested for toxins using the mouse bioassay. Other problematic dinoflagellates such as *Noctiluca scintillans* and *Karenia mikimotoi* (formally known as *Gyrodinium aureolum*) did not occur this year in appreciable quantities although high cell densities of *Noctiluca scintillans* were found off the west coast of Ireland during August last year.

Other toxic and potentially toxic species present off the west and north-west coasts of Ireland during 2001 consisted primarily of the dinoflagellates *Dinophysis* and *Protoperidinium* species. *Dinophysis* species responsible for Diarrhetic Shellfish Poisoning (DSP) were present intermittently in water

samples examined from Mc Swynes Bay throughout the year, with highest cell densities of >2000 cells.L^{-1} recorded in mid August. This was at a time when DSP toxin levels (1.00 µg.g^{-1}) were found to be above the regulatory limit (0.16 µg. g^{-1}) in mussels grown in the area. This dinoflagellate was not recorded in the surface samples taken in Clew Bay until mid July when the maximum cell density recorded was 80 cells.L^{-1} and DSP toxicity levels reached a maximum of 0.27 µg. g^{-1}. It is very difficult to establish if a relationship exists between the presence of the dinoflagellate *Protoperidinium* and the toxins responsible for Azaspiracid Poisoning (AZP). The reason for this is that the causative organism for this type of shellfish poisoning has not yet been fully confirmed. However, it is thought that at least one species from the genus *Protoperidinium* produces azaspiracid. Since the organism in question is heterotrophic in nature further research is required to elucidate what organism produces this toxin and if the causative organism can be monitored to provide more information on AZP toxic events.

Sample Collection and Analysis

Phytoplankton samples are taken on a weekly basis at specific sites (See Plate 1). Presently, samples at most sites are taken at discrete depths (usually the water surface) but it is hoped that integrated samples using a Lund tube will become the standard method of sampling next year. This method provides more information on the phytoplankton composition throughout the water column. After collection the samples preserved in Lugol's iodine are labelled and sent to the closest regional phytoplankton laboratory (Dublin, Bantry or Galway). At this point the samples are left to settle overnight in 25 ml sedimentation chambers. Quantitative counts are then carried out as quickly as is possible under an inverted phase contrast light microscope. Examples of toxic species are shown in Plate 2.

Phytoplankton Results from 2 Selected Production Areas

Temporal change in cell densities of diatoms and dinoflagellates in Clew Bay are presented for 2001 (Fig. 1). Concentrations of toxins present in mussel tissue and potentially toxic dinoflagellates are also plotted in separate charts against time for the 2 example sites (Mc Swynes Bay and Clew Bay; Figures 2-5). It seems that some relationship exists between the presence of the marine dinoflagellate *Dinophysis* and the biotoxins okadaic acid and its equivalents (Figures 2 and 4). There does not seem to be any clear relationship between the presence of AZP toxins and the genus *Protoperidinium* (Figures 3 and 5). However it is not yet been fully established what *Protoperidinium* sp. contain azaspiracid and if they can produce this toxin. Many of these species are heterotrophic in nature and may only serve as a vector of the toxin. Only surface samples were used for screening toxic microalgae at these times. It is therefore possible that thin layers of these organisms may have been present at depth during the toxic events. Integrated samples using Lund tubes should provide a clearer picture of the cell concentrations of toxic phytoplankton throughout the water column.

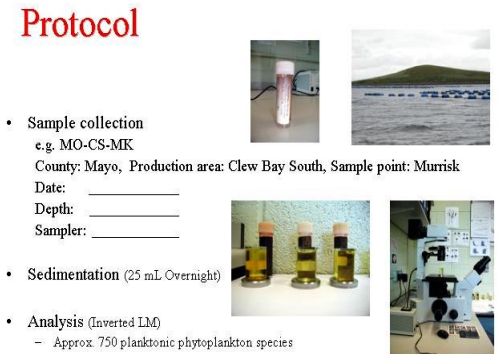

Plate 1. Sampling Collection and analysis

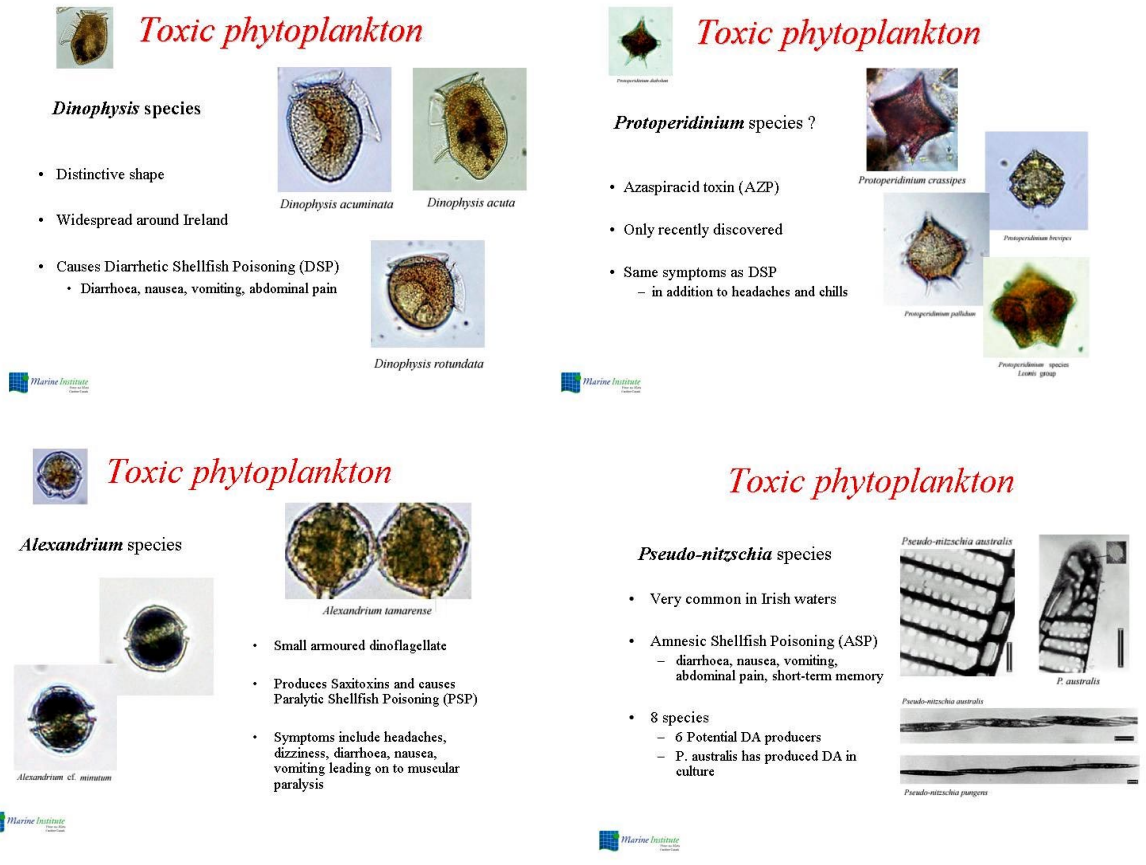

Plate 2. Examples of potentially toxic microalgae in Irish waters.

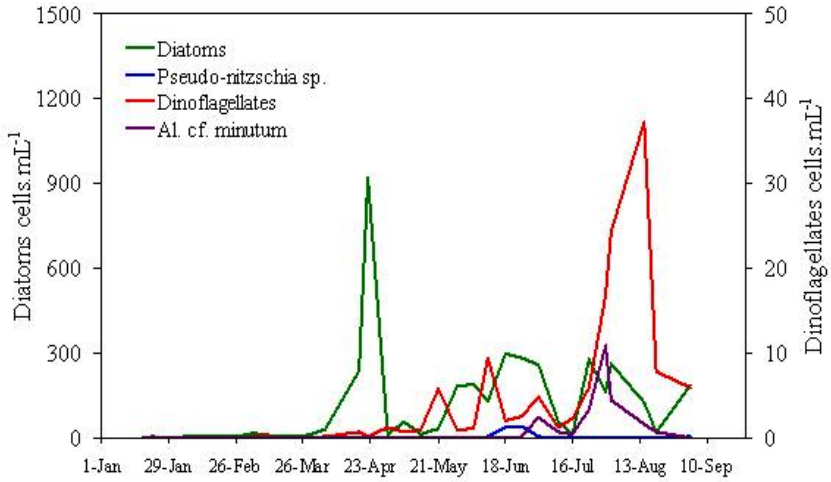

Figure 1. Phytoplankton abundance in Clew Bay in 2001.

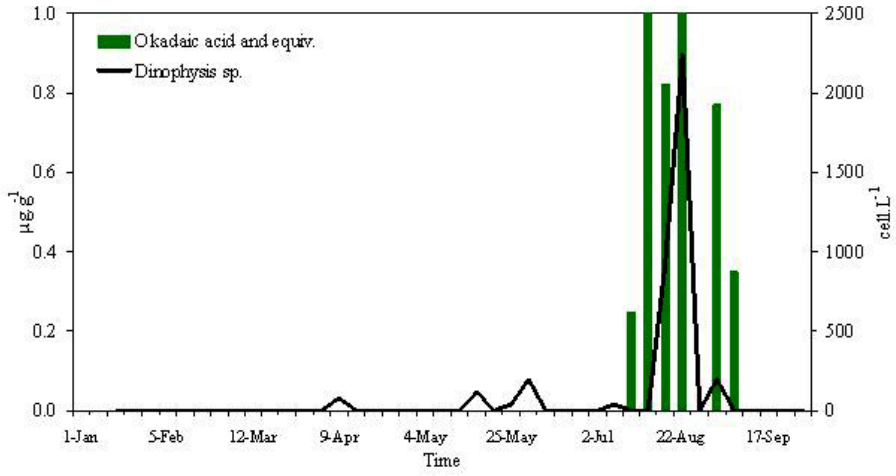

Figure 2. Toxic phytoplankton and chemical analysis in McSwynes Bay (trigger levels:0.16 µg g-1 okadaic acid and equivalents in the whole tissue).

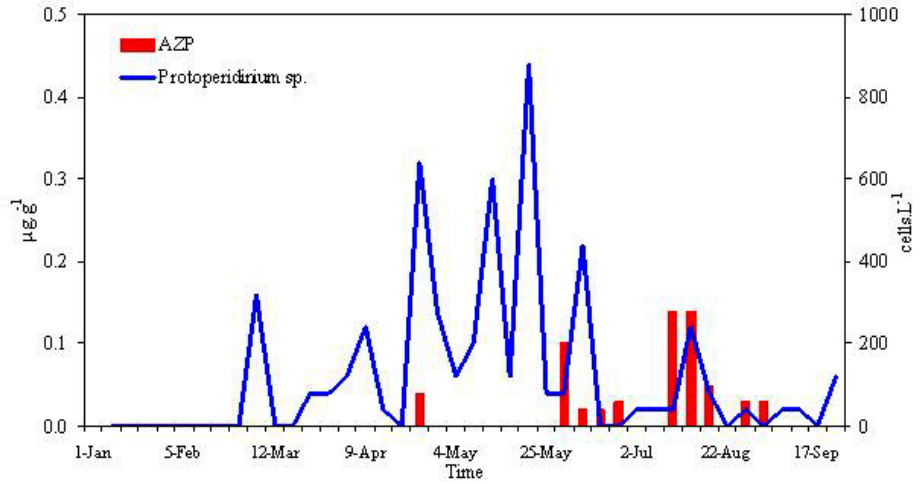

Figure 3. Toxic phytoplankton and chemical analysis in McSwynes Bay(trigger levels:0.16 µg g-1 azaspiracid acid in the whole tissue).

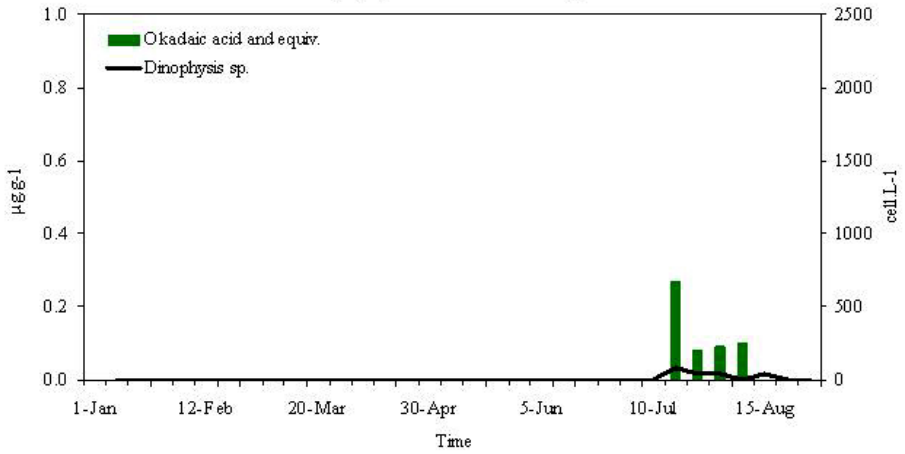

Figure 4. Toxic phytoplankton and chemical analysis in Clew Bay (trigger levels:0.16 µg g-1 okadaic acid and equivalents in the whole tissue).

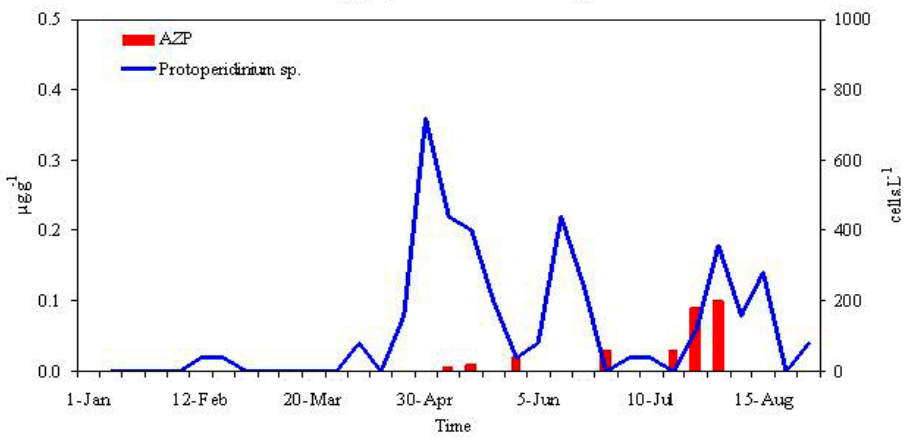

Figure 5. Toxic phytoplankton and chemical analysis in Clew Bay (trigger levels:0.16 µg g-1 azaspiacid in the whole tissue).

SUMMARY OF PHYTOPLANKTON MONITORING TRENDS IN IRELAND, 2001

Tara Chamberlain, Marine Institute

Following the changes in the Marine Institute Biotoxin programme this year, additional phytoplankton monitoring labs were set up in Galway and Bantry, West Cork. The lab in Bantry was functional by March 2001.The first joint step in the revamped monitoring programme involved the rerouting of samples to the regional labs. Each lab now has a specific geographical catchment area. This stage has progressed well following the industry's participation. The number of samples the south-west lab is receiving has risen from an initial 25/month to over 120/month.

One of the main features of the new regime is the continuous effort to respond to the requirements of the industrial and marine environmental changes. The current process is continually developing. To facilitate the changing needs, a constant review is ongoing. The volume of samples per week, per station is still fluctuating and will continue to do so until the updated sample location areas are implemented by the relative agencies. The relationships between the maximum and optimum number of samples per week per station are being studied. The turnaround time of the water samples is also being examined. The study of the relationships between the water sample being taken in the field, the time of arrival at the analysis laboratory, the length of time to analysis and reporting the data, are important factors in achieving the optimum turnaround time.

Current one of the main issues in the practicality of the monitoring programme, is the quality of the samples received. At present a significant proportion of the samples contain high amounts of debris, making it very difficult and often impossible to complete an accurate assessment of the to phytoplankton species present. The main sources of the problems with the samples may be due to the shallow depth of the sampling area and the taking of the sample at the surface of the water body. The possibility that the sample point itself may not be suitable, for various environmental reasons, must also be taken into account. The issue of the quality of the samples will remain a priority to the monitors.

Three sites were chosen within the south-west zone as examples illustrating the phytoplankton trends from January to September 2001. The sites chosen were outer Dunmanus Bay, N.Chapel in Bantry Bay and Kilmakilloge in Kenmare Bay. All sites represent areas of bivalve production. In order of magnitude, Bantry Bay has a capacity of 2000 tonnes, Kenmare 1700 tonnes and Dunmanus 500 tonnes (based on the highest B.I.M.annual returns in the last 3 years). So far this year N.Chapel has been open for approximately 20 % of the time, Kilmakilloge 48% of the time and Dunmanus 68%.

The 3 bays in the study are consecutively located along the south west coast. The phytoplankton diversity and biomass levels were examined and it was shown that all 3 bays followed the general spring, summer and autumn bloom and decline patterns expected for temperate waters. The relationships between the presence of *Dinophysis spp*. and levels of okadaic acid were examined in all sites. Both Dunmanus outer and N.Chapel appeared to show an apparent correlation between the increase of Dinophsis spp. cell numbers and a following increase in the levels of okadaic acid. This correlation could not be made in the Kilmakilloge site. This could be due to the lack of correlation or the different sampling technique used at this site. In Bantry and Dunmanus, samples are taken beside the mussel lines, to a depth of 10m using the Lund tube method. The sample from Kilmakillogue is a surface sample only. The relationship between the increase of cell numbers of *Protoperidinium* spp. and AZP levels was also studied. There did not appear to be any clear correlation in any of the 3 subject sites. This could be related to the fact that the *Protoperidinium* cell numbers related to all species present and not to specific species currently targeted as potentially toxic. Further studies are continuing.

In conclusion, 3 basic points were highlighted as factors that are significant in improving the quality of the monitoring programme (Plate 1). The first issue relates to the debris content, which is present in some samples, and the immediate need to reduce this problem. This is a function of both the site and the sampling technique. The introduction and implementation of the Lund tube should enable better sample quality and improved sub sampling representation. Finally the correct preservation and availability of live samples if necessary was identified as another basic issue related tom the continual improvement of the phytoplankton monitoring programme.

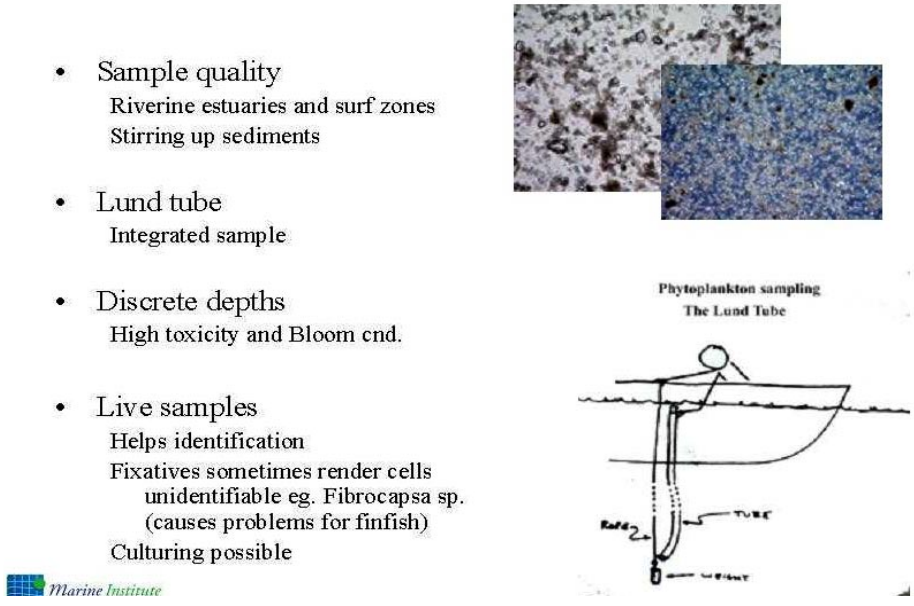

Plate 1 Recommendations

UPDATE ON OCEANOGRAPHY AND PHYTOPLANKTON IN IRISH WATERS

Robin Raine[1], Glenn Nolan[1], Juan Brown[2] and Liam Fernand[2]
1. Martin Ryan Institute, National University of Ireland, Galway
2. CEFAS, Lowestoft Laboratory, United Kingdom

Summary
Much progress has been made in recent years in determining the seasonal circulation patterns in the Celtic Sea and along Ireland's west coast (Raine et al. (1998), O'Boyle et al. (2000), Brown et al. (in press)). One of the key findings to date is that in summer there are persistent pathways that transport phytoplankton and passive particles around the Celtic Sea and the western Irish continental shelf. There has been much debate as to whether this is a continuous system of currents from St. Georges Channel, via the Celtic Sea to North of Ireland or not. Some of the work presented here dispels any doubt about whether the circulation is continuous (in summer).

Figure 1 shows the stations at which phytoplankton samples were taken during a collaborative cruise involving the Biotoxin Team of the Marine Environment and Health Services Division of the Irish Marine Institute, NUI Galway and the Centre for Environment, Fisheries and Aquaculture Science, U.K.. In conjunction with bottle samples taken at discrete depths (marked as blue dots), the cruise gathered the most extensive set of hydrographic measurements (temperature, salinity, fluorescence) in this shelf region to date. This was possible due to the use of an undulating vehicle, known as Scanfish, that "yo-yos" astern of the ship.

The cruise builds upon previous efforts in the Celtic Sea (1998) and along the west coast of Ireland (1999) using satellite-tracked drifting buoys as a means of establishing the mean circulation in summer. The drifters are drogued at 35-40m so that the density-driven circulation is represented by the drifter tracks and not the near surface circulation that is more likely to be caused by wind forcing. Figure 2 illustrates the anti-clockwise circulation observed in the Celtic Sea and the South to North circulation along Ireland's west coast.

From Scanfish measurements the speed of the density-driven flows can be calculated and can be observed in figure 3 extending from Fastnet Rock to Malin Head as a continuous current system. A significant feature in the 2001 data set is the degree to which the salinity of the water column is fresher that observed in 2000. Figure 4 illustrates this by showing water generally of salinity ≥ 35.3 in the upper diagram while water ≤ 35 pervades the section along latitude 53 north in 2001. This is most likely due to the extensive run-off from excessive rainfall in the autumn and winter of 2000 freshening the coastal ocean.

A reasonable understanding of the summer circulation along the Irish shelf has been gained through fieldwork since the early 1990s yet a critical question is how variability offshore effects aquaculturally sensitive bays inshore. In an attempt to study this, several temperature sensors were

installed at aquaculture sites in Bantry Bay in summer 2001 (Figure 5). The sensors were placed every 7-8m through the water column to the seabed to gain insight into processes at work within the bay. The data presented herein show the sequence of events from May 15th to August 8th 2001. The upper plot in figure 5 shows data from the Roancarraig site on the north shore of Bantry Bay while the lower panel shows data from the Gearhies site on the bays southern shore.

At the beginning of the record the water column is well mixed with no evidence of stratification in the bay. As summer progresses, stratification begins and is apparent as 14º C water in the surface layers. On day 165 (June 16th 2001) a cold pulse of water is observed at depth at both sites representing a 4º C temperature drop in 12 hours. Toxicity in shellfish within the bay occurred approximately 7 days later with the closure of many of Bantry's aquaculture sites as a result. There are several other cold water episodes during the period for which data are available, most notably around July 5th and July 18th.

Perusal of the meteorological data from Valentia Observatory nearby suggests a strong link between wind forcing and the observed temperature fluctuations within Bantry Bay. In figure 6 the axial wind (running parallel with the bay) is plotted against the bottom temperature record from the Gearhies site. The predominant wind in this region is typically from the south-west. The data show that when there is a reversal in the predominant wind to an easterly direction, a resultant drop in bottom temperature is experienced.

In the future it is possible that some predictive capacity can be gained from the real-time measurement of ocean temperature and wind in Bantry Bay. This work also demonstrates a useful means of deploying probes on existing aquaculture structures such as salmon farms and mussel lines to gather crucial environmental data related to harmful algal events around the Irish coast. The full co-operation of the growers and producers as well as links between different scientific disciplines is the key to achieving this.

Acknowledgements:
We thank the officers and crew of RV Celtic Voyager and Corystes for their assistance. Thanks also to Paul Nee, Mike Sammon and Liz Abbott for allowing access to aquaculture sites in Bantry. Sincere gratitude to Kevin O'Mathuna of Met Eireann for the Valentia wind data. The MEHS division of the Marine Institute funded the Irish cruise work component of this research. The Celtic Sea component was funded by MAFF,U.K.

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O'Boyle,S., Nolan,G. and Raine,R. (2001) Harmful phytoplankton events caused by variability in the Irish coastal current along the west of Ireland. Proceedings of the 9th Annual Harmful Algal Bloom conference, Tasmania, Australia, February 2000.

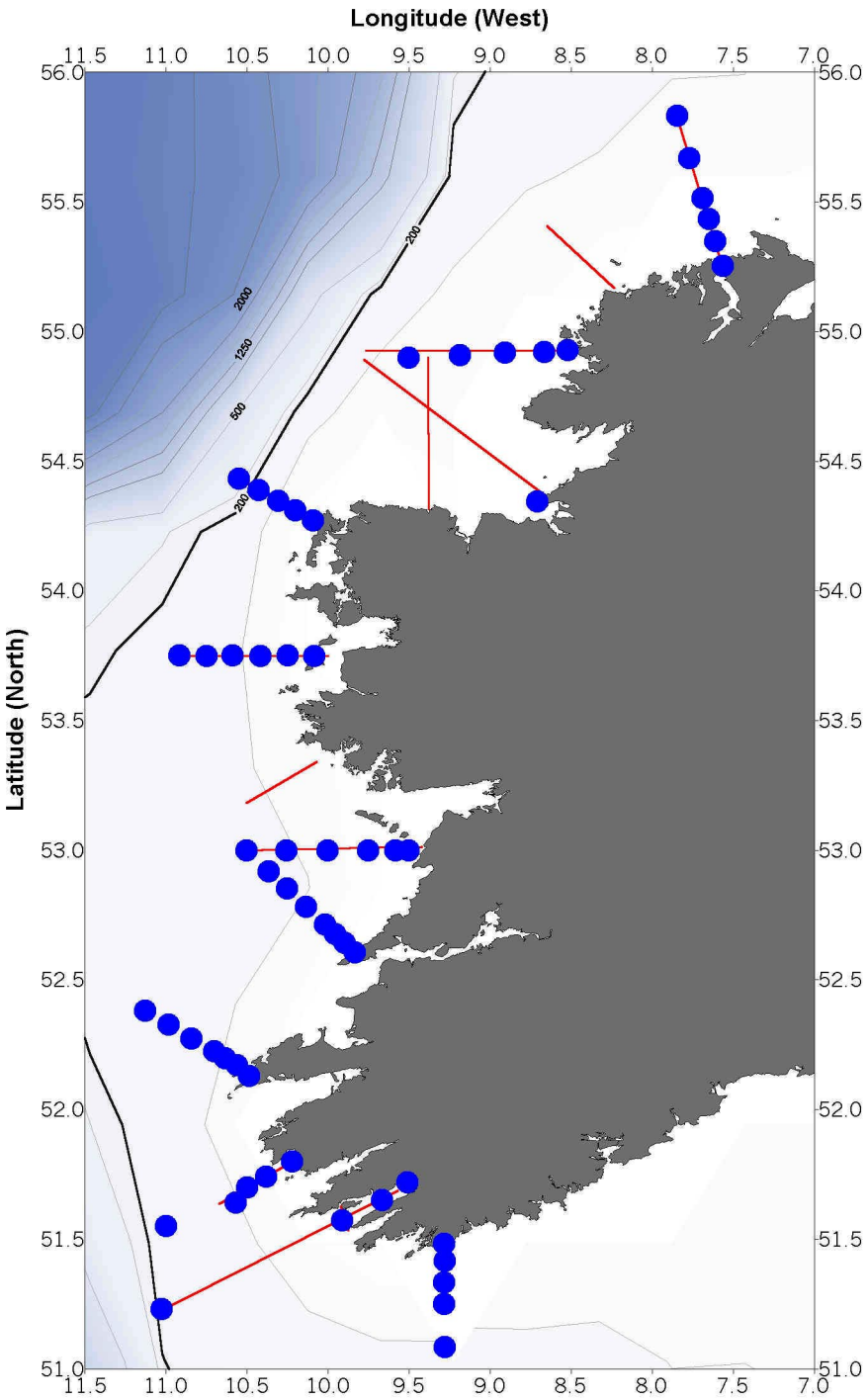


Figure 1. Study area for joint MEHS, NUIG, CEFAS cruise on RV Celtic Voyager, 2001. Red lines denote Scanfish profiler transects while blue dots denote phytoplankton bottle samples at several depths using a CTD Rosette sampler.

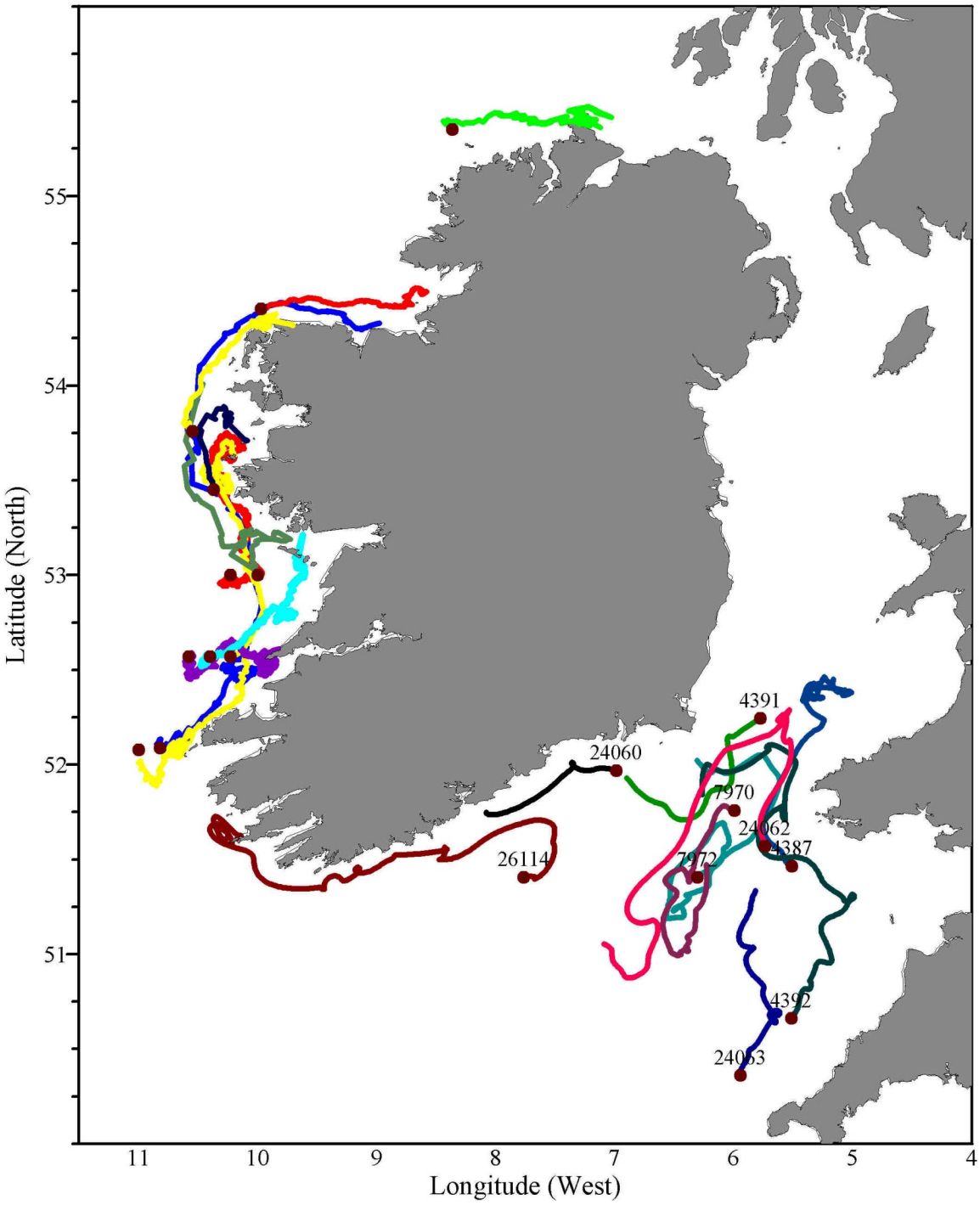


Figure 2. Combined drifter tracks from CEFAS (1998) and NUIG (1999,2001) data archive. Brown dots denote release positions of the drifters. Celtic Sea drifters have drifter ID on plot also.

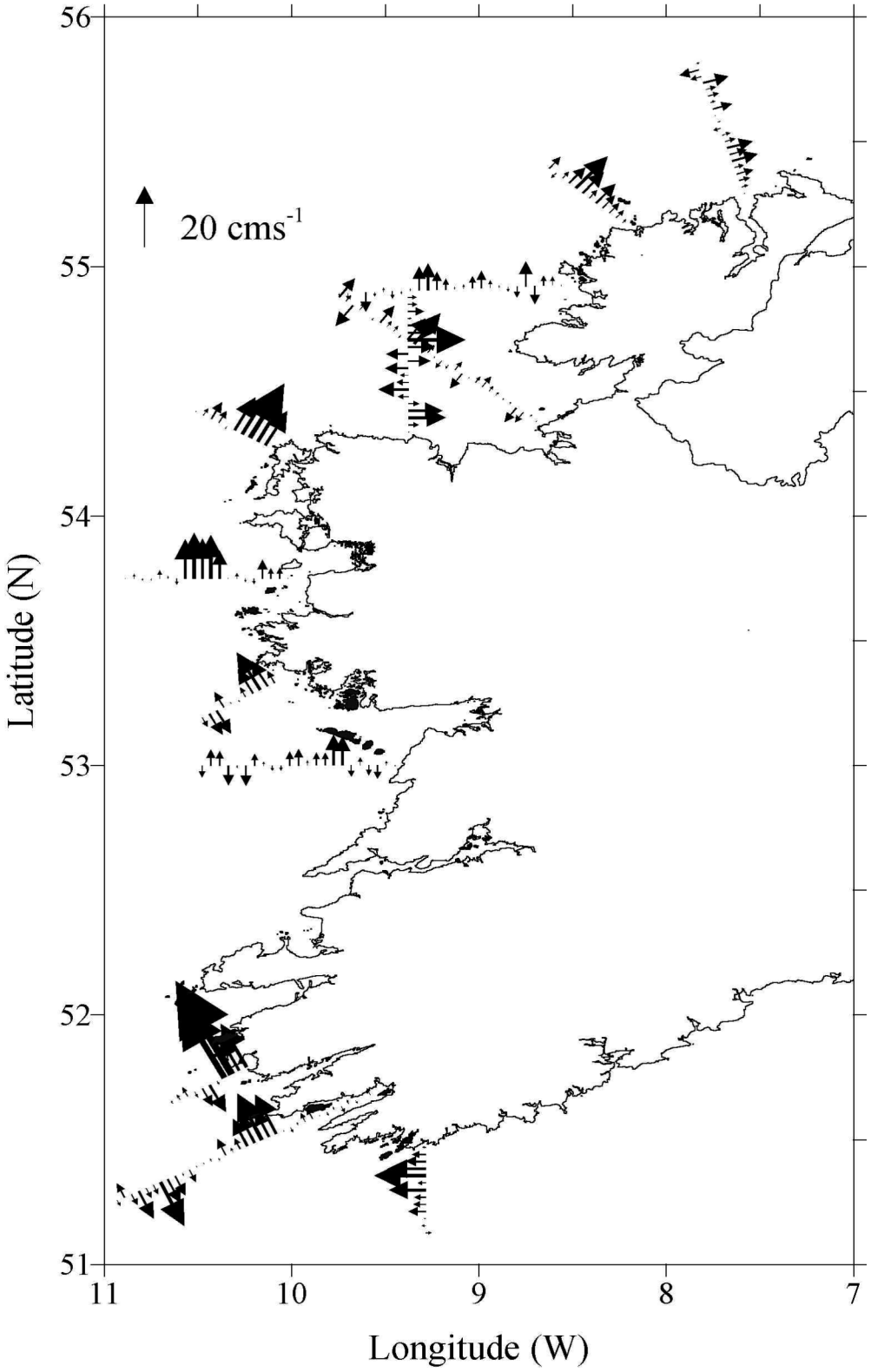


Figure 3. Geostrophic velocities calculated from Scanfish data in 2001 showing the predominant northward flow along Ireland's west coast during the summer season.

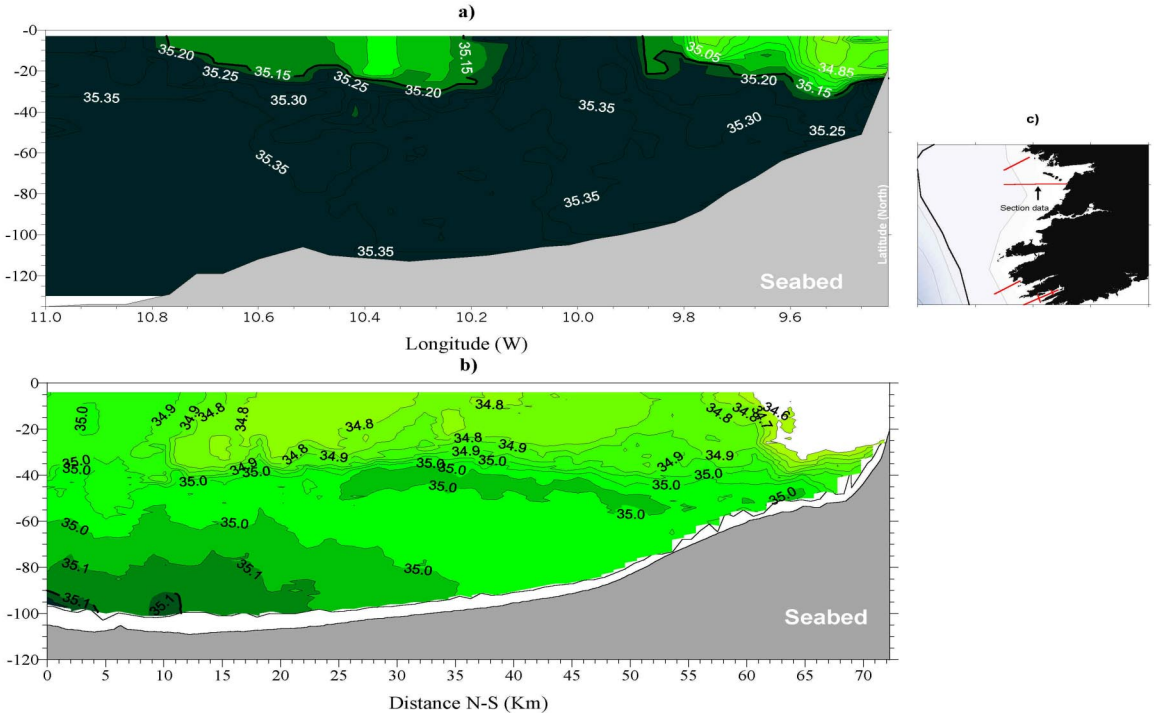


Figure 4. Variability in the observed salinity structure west of the Aran Islands (see plot 4c). a) salinity along the Aran section in 2000 showing salinity ≥ 35.3 while b) shows a much fresher coastal ocean in 2001 along the same hydrographic section. Colour scaling is the same for each plot.

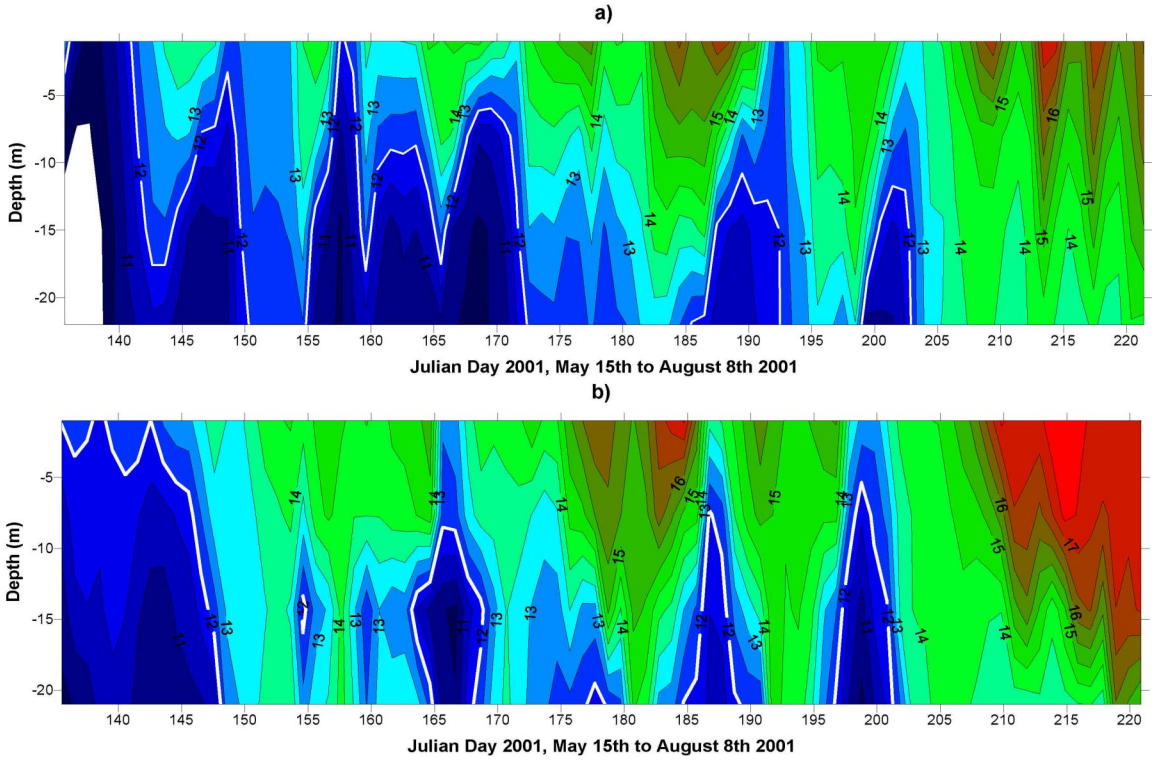


Figure 5. Temperature data from TidBit loggers in Bantry Bay during summer 2001. a) Roancarraig site (northern shore) and b) Gearhies site (southern shore).

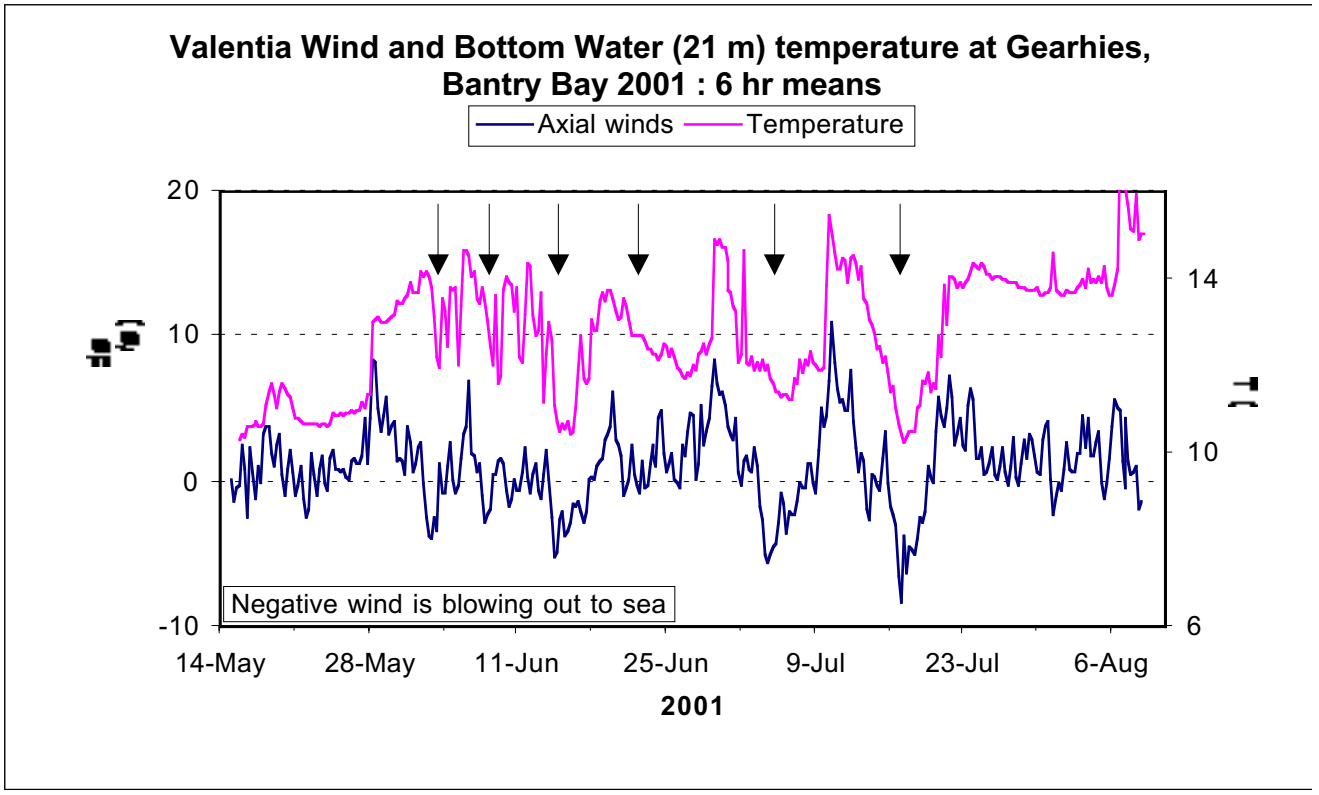


Figure 6. Comparison of bottom temperature time series at the Gearhies site with the axial component of the wind derived from Valentia wind record. Correlation between decreased bottom temperature and a reversal of the wind to an easterly direction is evident from this data.

OVERVIEW OF CURRENT PHYTOPLANKTON RESEARCH IN IRELAND

David Clarke, Marine Institute

Current phytoplankton methods for monitoring programmes concentrate on the identification and enumeration of species (mainly dinoflagellates and diatoms) with particular attention to known or suspected toxin producing / nuisance species harmful to shellfish and finfish.

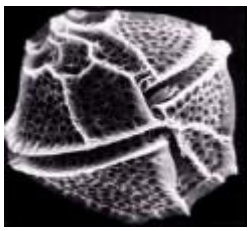
Whilst phytoplankton monitoring is a valuable part of any biotoxin-monitoring program, further valuable information can be acquired through the examination of sediments for the presence of dinoflagellate cysts. This information can be used as another part of the jigsaw in part in determining and explaining periods of shellfish intoxification in aquaculture intensive producing areas.

Certain dinoflagellate phytoplankton species have a known permanent cyst (resting) stage. These cysts are formed in response to unfavourable conditions such as reductions in temperature and light intensity. These cysts can remain viable for long periods of time, until such a time favourable conditions return and the motile stage is formed once again. Typically the majority of cysts rest in the top 1 – 5 cm of sediment.

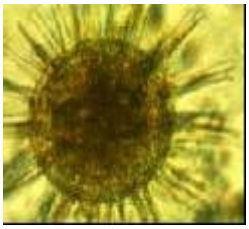
Resting cyst populations of the PSP causative species *Alexandrium tamarense* have been shown to be present in sediments from Cork Harbour. Hence intoxification of shellfish due to the hatching of these cysts have led to periodic closures of shellfish from being harvested from this area for the last few years during the summer months.

Sediment samples taken in 1997 from Bantry Bay have shown large viable cyst populations of *Lingulodinium polyedrum*. (Plate 1). This species has recently been shown to produce Yessotoxins (YTXs) in Italy. To determine if this was true in relation to Irish waters, sediment samples from Bantry Bay were taken in 2001. The cysts were isolated and hatched under laboratory conditions. When analysed the cells were found to contain a low concentration of YTXs. Total YTX production was estimated at 0.3pg/cell. Therefore it is further estimated that blooms of over 200,000 cell/litre are required to cause shellfish intoxification of 100µg/100g TT. Historical phytoplankton data has shown that these levels have been exceeded in past years with counts in some sites within the Bantry Bay area exceeding 500,000 cells/litre. Further confirmatory analysis of laboratory cultures is desirable through LC-MS techniques to further quantify these estimated levels and to determine which analogues of YTXs are present.

This approach of examining toxin production levels of phytoplankton species provides more detailed information in determining the threshold levels of phytoplankton species present to initiate shellfish flesh testing.



SEM *Lingulodinium polyedrum* – motile stage



Lingulodinium polyedrum *cyst*

Plate 1. *Lingulodinium polyedrum* found in Bantry Bay sediments in 1997.

Protoperidinium crassipes has recently been linked to Azaspiracid (AZP) production. However, at present it has not been established whether all species of *Protoperidinium* produce AZP. One way to investigate this is to examine motile phytoplankton populations with analysis via LC-MS. Over 60 net haul samples were taken this year from the North-West down to the South Coast in July/August 2001 and these are to be examined to further investigate the links between AZP levels and phytoplankton species present. Live samples from these net hauls were also cultured to a certain degree of success; these species include *Prorocentrum micans, Scrippsiella sp., Pseudo-nitzschia sp., Coscinodiscus sp.,* and *Thalassiosira sp.* Other species observed include *Protoperidinium sp., Dinophysis sp., & Ceratium sp.*

A second way is to investigate cyst seedbed populations (methods previously outlined above). Over 20 sediment samples have been taken this year in Donegal Bay, Baltimore and the entrance to Bantry Bay. Analysis of these sediments is currently ongoing. The aims are to identify the cyst species present, to isolate and culture the species to motile stages with particular attention to *Protoperidinium sp*..

The lack of standards world wide for the analysis of shellfish via chemical methods is a major problem, particularly AZPs and YTXs. One method is to isolate toxins from contaminated shellfish, and the second is to isolate toxins from phytoplankton cultures. Both of these methods form the basis of two separate projects, which have commenced in the Biotoxins Unit of the Marine Institute with external partners in producing and providing AZA standards.

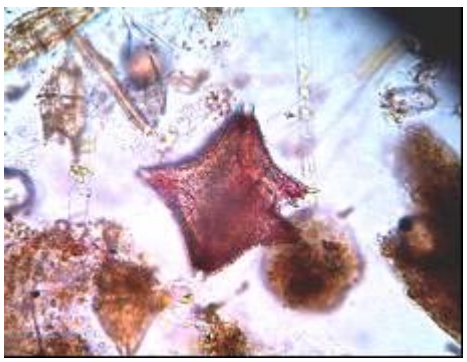
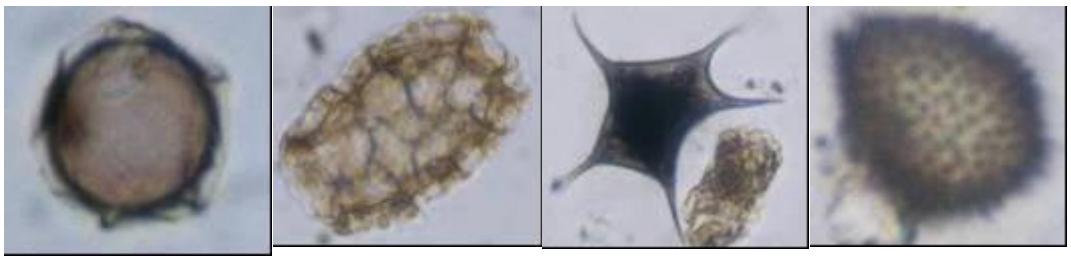


Plate 2. *Protoperidinium crassipes* found in net hauls taken during July / August 2001 – Celtic Voyager Cruise

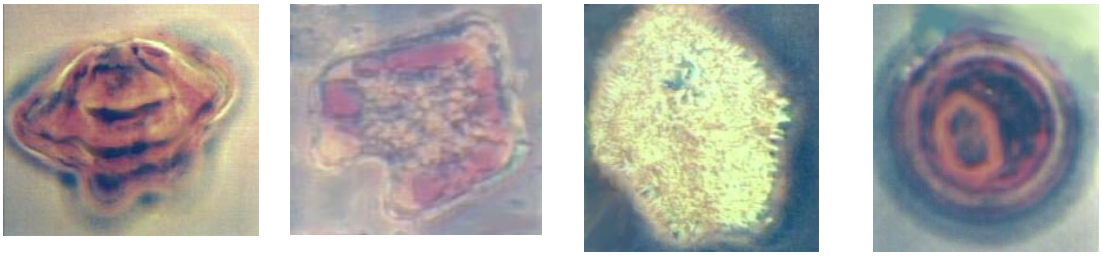


a)*Diplosalis sp.* b) *Polykrikos sp.* c)*Protoperidinium sp.* d)*Scrippsiella sp.*

Plate 3. Other cyst species found in Bantry Bay sediments in 2001.

a) *Protoperidinium sp.* b) *Dinophysis sp.* c) *Ceratium sp*.

Plate 4. Other species observed from net haul samples taken July/August 2001



Protoperidinium leonis *Protoperidinium oblongum* *Protoperidinium pentagonum* *Protoperidinium subinerme*

Plate 5. Cysts of *Protoperidinium sp*. previously found in Irish coastal sediments

ONGOING RESEARCH ON TOXIC ALGAE

Dr. Cilian Roden

In 2001 continuing closures of shellfish beds caused great commercial difficulties to mussel producers and exporters. As part of a programme to alleviate the impact of these closures, Bord Iascaigh Mhara funded investigations into ways of protecting shellfish farms from the impact of toxic blooms. Here I report on work in progress undertaken at the request of Bord Iascaigh Mhara.

Plankton collection programme; It is essential to discover the causative organism of AZA toxicity. In September 1999 and 2000, plankton samples collected off the south-west coast were shown to be toxic. In 2001 a further series of collections were undertaken at several points along the Irish coast including water south of Baltimore Co. Cork, water south-west of the Aran Islands and Killary Harbour. Samples were collected from March to September. The plankton hauls were subsequently analysed at Cork Institute of Technology by Dr Kevin James and his colleagues. To date the only samples found to contain AZA were collected off Baltimore and Aran in late summer. These samples were collected from stratified water at a depth of 80m with surface temperatures of 15^{o}C to 17 ^{o}C. The plankton off Aran in late summer was very sparse but a large proportion of the net plankton consisted of *Protoperidinium* species. A pure *Protoperidinium* sample was prepared by isolating cells using a pipette. This sample was frozen and subsequently analysed. It proved positive for AZA. A second sample was then collected in mid September. Three separate species of *Protoperidinium* were then isolated. *P. crassipes*, *P. depressum* and *P. divergens*. These samples are now being analysed; while *P. crassipes* has been shown to contain AZA, the other species are still under examination.

Monitoring toxic algae through the C.L.A.M.S. committee system. Killary Harbour C.L.A.M.S. committee has initiated a monitoring scheme for toxic algae. The purpose of the scheme is to allow growers to gain a clearer understanding of the local factors that determine toxicity in the Harbour. Plankton collection has been undertaken by Richard West and Kevin Lydon and plankton examination has been undertaken by Tómas Burke and Cilian Roden. To date the most interesting results include data that show that the inner Killary contains a different algal population than the outer Killary and that

few toxin producing algae occur there. A second finding is that dinoflagellates, like mussel larvae are not evenly distributed in the water column but occur as narrow bands often near salinity discontinuities. In late summer a twenty-fold difference in concentration was recorded between a plankton band at 5m and surface samples. It is hoped that these very large differences in plankton concentration might allow mussels nearing harvest size to be stored in such toxic alga free areas. However further data must be gathered to allow this proposal to be tested.

MARINE BIOTOXIN MONITORING AT THE PUBLIC ANALYST'S LABORATORY, GALWAY

Andrew F. Flanagan, Caroline M. Lardner, Louise Mannion and Padraig Burke
Public Analyst's Laboratory, Western Health Board College Hospital, Galway.

The Public Analyst's Laboratories, based in Dublin, Cork and Galway, are Official Food Laboratories within the Department of Health/Health Board Food Control system. The Galway laboratory provides a regional chemical testing service to the Western, North-Western and Mid-Western Health Boards (a microbiological service is provided by the Official Food Microbiology Laboratories). The laboratory is divided into three main sections; food, water/environment and medicines/toxicology.

Marine biotoxin analysis is carried-out in the food section, together with monitoring of various foodstuffs for other toxins and contaminants etc. The pre-planned aspect of the testing is as per a Regional Programme agreed between the laboratory, the Environmental Health Officers (EHO's) and the Food Safety Authority of Ireland (FSAI). The Regional Programmes form an element of the service contracts between the F.S.A.I. and the Health Boards. A series of meetings involving the F.S.A.I., the Department of Marine/Marine Institute and the Western Health Board defined the agreed role of the Health Boards in biotoxin monitoring.

Sampling is undertaken by the EHO's, who collect shellfish largely from retail and catering premises. Processed and imported products are included in the monitoring, together with raw, Irish produce. Currently samples are tested for DSP-, AZP- and ASP toxins; DSP and AZP testing is by LC-MS, and ASP analysis is by a HPLC method. The monitoring is at a relatively low level (ca. 200 samples per year) and it is being extended from the May to October period to throughout the year. The table below summarises results of analysis:

Year	No. of samples	Toxins tested-	No. of "Unsatisfactory" Samples	Method(s)
1996 98	41	DSP	0	ELISA
1999	100	DSP	0	ELISA
2000	173	DSP/AZP	1	ELISA+CELL-ASSAY
2001	193	DSP/AZP/ASP	1	LC-MS+HPLC

Although the number of samples tested is small, the results indicate that, despite the extensive problems of bay-closure, the production-level control, operated by the Department of Marine/Marine Institute in conjunction with shellfish farmers, is effective.

The evolution of marine biotoxin monitoring programmes in New Zealand

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A brief history of marine biotoxins in NZ

Prior to 1993 there was very little expenditure (<$NZ 15,000/year) on research or monitoring of marine biotoxins. This situation changed after the austral summer of 1992/93 when there was a major bloom event on the north-east coast of the North Island, resulting in the contamination of shellfish with neurotoxic shellfish poisoning (NSP) toxins. This led to a couple of hundred people suffering some ill effects. A combination of lack of analytical experience and the use of an extraction procedure (using 1.0N HCl) which caused large numbers of false positive mouse assays, resulted in an unnecessary, prolonged, and damaging nationwide closure of all shellfish harvesting.

As a result of this experience, substantial resources were made available by the government to carry out research on the causes of bio-toxin contamination and development of appropriate testing methods, and to establish a comprehensive monitoring programme based mainly on mouse assays (Fig. 1).

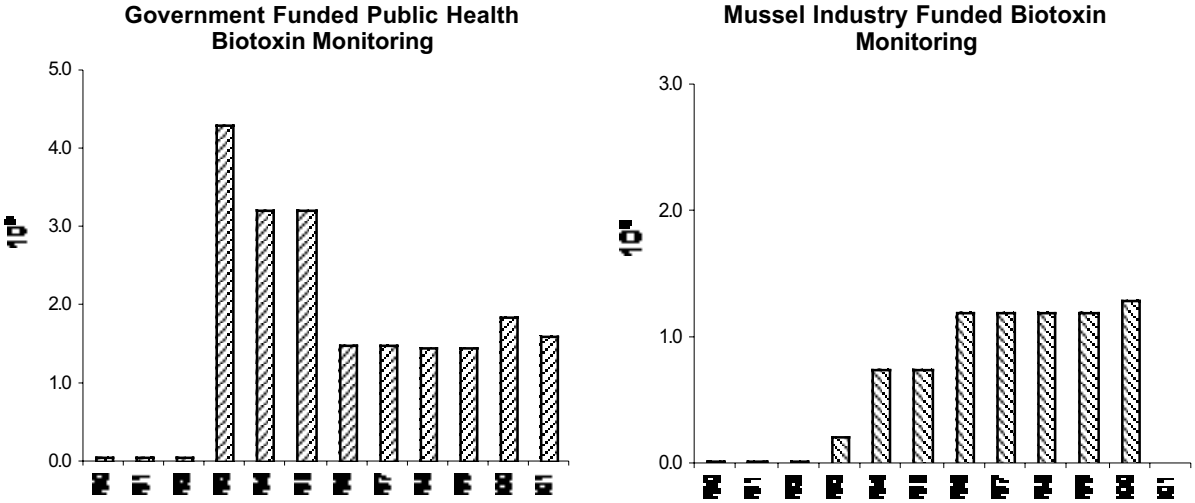

Figure 1. Estimates of the expenditure by government and the shellfish industry on marine bio-toxin monitoring

Between 1993-1996 an increasing proportion of the costs of monitoring in commercial areas were borne by the industry. Also over this period significant savings were made by the introduction of phytoplankton monitoring as the first tier of a staged response, thereby providing an early warning capability and reducing the number of routine flesh tests necessary. In 1996 the entire financial responsibility for carrying out monitoring in commercial growing areas was assumed by the industry. New Zealand currently exports about

$NZ 230 million worth of shellfish products (>85% are Greenshell mussels) to over 60 countries.

New Zealand Biotoxin monitoring organisation
There are two complementary programmes in operation

- The Public Health Programme, which monitors shellfish in non-commercial areas, where the public harvest shellfish for their own consumption. This is operated and funded by the New Zealand Ministry of Health (MOH).

- The New Zealand Shellfish Industry Programme is 100% funded by the various commercial enterprises involved and is regulated according to procedures promulgated by the New Zealand Ministry of Agriculture and Forestry (MAF), Food Assurance Authority through the "NZ Marine Biotoxin Management Plan". The various shellfish cultivation and harvesting areas throughout the country are managed at a local level by "Delivery Centres" (e.g. Marlborough Sounds Quality Assurance Programme). The delivery centres (there are 27 of these) are funded by levies on marine farming license holders.

The Technical Committee of the NZ Marine Biotoxin Management Board that includes representatives from MOH, MAF and Industry (with input from scientists) provides technical advice when revisions of the Management Plan are required. There is some sharing of costs and data between the industry and MOH programmes.

Shellfish monitoring
Since the inception of the shellfish monitoring programme, tests have mainly been based on the use of mouse assays. Most samples have been routinely screened for PSP toxins using a 0.18N HCl acid extraction, and for the lipophilic DSP and NSP group toxins using an acetone extract with a dichloromethane clean-up step (Table 1).

Table 1. Current and future methods of assay and analysis

Toxin	Current methods	Future methods	Back-up methods
PSP	Mouse assay	Mouse assay/ Immunoassay	HPLC, cell assay
ASP	LC-MS/MS	LC-MS/MS	HPLC
DSP/NSP screen	Acetone/DCM extract mouse assay (3 mice)	LC-MS/MS	Mouse assays PP2A, HPLC, immunoassays
NSP (confirmation)	Ether extract (5 mice)	Phytoplankton probes* LC-MS/MS Immunoassay?	Mouse assays, cell assays
DSP-'Lipotox' confirmation	ELISA (OA, DTX1) PP2A (OA, DTXs) HPLC (YTX) LC-MS/MS (all)	Not needed	Not needed

* Interim

In the event of a DSP/NSP screen test returning a positive result, the identity of the toxins has usually been confirmed using an immunoassay test (ELISA) for DSP toxins, or a repeat diethyl-ether extraction for the confirmation of NSP group toxins. Occasionally other test methods (HPLC, LC-MS, PP2A) have been used for confirmation of the identity of contaminants such as okadaic acid, yessotoxins and pectenotoxins. Domoic acid (ASP-toxin) has been routinely screened using HPLC analysis.

The number of individual shellfish flesh tests carried out annually has fallen from a high of >15,000 in 1994 to about 3,000 in 1999. In most years there has been a very low rate (<4%) of positive tests and only a minor proportion of these (<30%) have been over the regulatory limits. On the other hand when sampling has focussed on a particularly important event (e.g. the *Gymnodinium catenatum* bloom during 2000-2001) a high proportion of some types of positive tests (e.g. PSP assays) have occurred. In a minority of analyses, there has been a recurrent problem of lipid positive mouse assays in the absence of any confirmatory evidence from phytoplankton data or follow up chemical tests (e.g. LC-MS). On some occasions these apparent 'false positive' assays have resulted in sizeable financial losses to producers.

Phytoplankton monitoring

Over the 7-8 years that phytoplankton monitoring has been practised in NZ it has proven itself to be a reliable and cost effective supplement to shellfish flesh testing. Long term data series have shown good correlation between phytoplankton and toxin analyses for all toxin groups and the onset of toxicity has been predicted in many cases. Harvesting closure and opening decisions however are usually based on the results of flesh tests only. The major

limitation to phytoplankton monitoring relates to sampling problems in remote locations.

- More than 70 sites/week are sampled nation-wide, 3,300 samples were analysed in the year 2000
- Careful sample site selection is important (the site should be representative of a larger water body). Some locations may never be suitable for phytoplankton monitoring
- Sampling is preferably carried out from boats (to avoid shoreline effects) where possible, sampling personnel need to be properly trained
- Most routine sampling is carried out using a 15m (Lund) tube
- Discrete depth samples are taken when required (e.g. to quantify cells in subsurface layers)
- Live and preserved samples are routinely collected (live samples assist with species identification)
- Most samples are examined and counted after sedimentation in 10 ml Utermohl chambers, results within 24 hours of sample receipt are guaranteed
- When necessary phytoplankton IDs are confirmed by various methods (e.g. Calcofluor staining, electron microscopy)
- A comprehensive list of proven and possible toxin producers in NZ waters has been compiled and appropriate trigger levels at which flesh testing is initiated have been established
- Phytoplankton analysis is a demanding job, staff turnover and a need for back up means continual training of analysts is necessary (the Cawthron phytoplankton laboratory has ISO 17025 accreditation)
- Whole cell DNA probes for toxic *Pseudo-nitzschia* (IANZ accredited to ISO 17025) are routinely used for definitive identifications, genetic probes for *Alexandrium* and *Gymnodinium* will be available soon and semi-automated sandwich hybridisation format probes are being trialled

The high level of sampling and comprehensive analysis undertaken throughout the entire country between 1993-1996, and the reduced but still extensive programmes that have been maintained since, have provided an extremely valuable perspective on the real nature and incidence of biotoxin contamination. We now know that almost all species of toxic phytoplankton reported to cause shellfish poisoning exist in New Zealand waters, and most known toxin groups (with the exception of spirolides and azaspiracids so far) are represented. Minor, localised incidents are not uncommon (and in some cases are semi predictable) but cause few problems, whereas major extensive events occur infrequently but have the potential to be quite harmful if not first detected through monitoring. There has been a very low reported incidence of human illness despite the widespread occurrence and variety of biotoxin contamination events. In part this is no doubt due to the success of the monitoring programmes. However it is also due to the usually rather low concentration of potentially serious toxins (e.g. PSP toxins) encountered and the low specific toxicity of some compounds (e.g. yessotoxins and gymnodimine) when consumed.

Towards a chemistry based shellfish screening programme

Research and technical developments have at last made the prospect of largely replacing the mouse assays with alternative methods a reality and during 2000-2001 a determined move towards the adoption of new methods for shellfish flesh testing in New Zealand was made. This has mainly been prompted by, the high cost, slow speed and occasionally questionable results obtained by the use of mouse assay for the lipid soluble toxin groups (DSP, NSP, YTXs, etc.). A culture of innovation within the shellfish industry, a desire for a world leading "state of the art" monitoring system, the ethical dilemmas inherent in the continued use of animal testing, and good scientific support were also important factors in bringing this about.

Any new test methods that are adopted need to:

- reliably detect and quantify all marine bio-toxins occurring in New Zealand
- minimise the use of mice
- be quick and have improved turnaround times
- be cost effective
- be internationally accepted so market access is maintained

A variety of alternative methods have been evaluated including immunoassays, cell based assays, enzyme assays, HPLC and liquid chromatography with mass spectrometry detection (LC-MS). Various configurations of these tests may ultimately be used to screen for particular toxins groups but LC-MS analysis has been determined as the method of choice for screening for the "Lipotox" compounds. It is unlikely that mouse assays will be completely eliminated in the medium term especially for the water soluble PSP-toxin group. The programme will continue to evolve using the optimal mix of technologies to provide the most effective risk management

In August 2000 the NZ Marine Biotoxin Technical Committee (MBTC) approved the concepts proposed by the Cawthron Institute for the introduction of new technologies. In October 2000 the Cawthron Institute purchased a MicroMass Quattro Ultima, high-resolution LC-MS/MS system, which will be the heart of the new look monitoring programme. This machine is identical to that recently purchased and successfully operating at the Marine Institute, Dublin.

Why choose LC-MS/MS?

- Definitive ID of toxins in complex mixtures, no need for confirmatory tests
- Automated multi-toxin screening
- Rapid and cost effective, same day results possible
- No false positives, no interference from compounds active in bioassays but of no relevance to human health
- Reduction in the use of laboratory mice
- Powerful research tool, provides new information about the source, chemistry, toxicology and metabolism of toxins

LC-MS/MS method validation

New methods of analysis must be rigorously evaluated before they can be used for routine regulatory testing. MAF-Food Assurance Authority has issued

guidelines outlining the validation requirements and the procedure for the introduction of new test methods (Burrow and Seamer 2001). The guide is based on several internationally recognised protocols and new methods have to meet a set of defined validation performance characteristics including: Quality Control Procedures, Accuracy, Precision, Sensitivity, Ruggedness, Working and Linear Ranges and Matrix effects.
New test methods need to be validated against all tissue types and organisms of interest. Final approval to use a new method is given by MAF National Manager Seafood upon recommendation from the MBTC. An LC-MS/MS method for domoic acid (ASP toxin) has been validated and is now in routine use, validation of methods for the DSP, YTX and PTX group toxins is expected this year.

Standards and reference materials
Quantification of toxins by LC-MS/MS requires calibration of the instrument with analytical standards consisting of precise amounts of highly purified toxic compounds and reference materials (i.e. shellfish homogenates) which contain defined amounts of the toxins in a natural non-pure state. Standards and reference materials for most of the toxins are not commercially available and acquiring these materials is a challenge. Fortunately sufficient quantities of the major parent compounds have recently been obtained from natural blooms and the culture of toxic algae and in house standards and reference materials for these have been made. This allows accurate quantification of the parent compounds (e.g. YTX, PTX-2, Gymnodimine) and semi-quantification of related toxins and metabolites (e.g. PTX2-SA, 45 OH-YTX).

Data management
A new interactive, web based, GIS linked data base is under development. This will allow users to access information by clicking on locations on a map (e.g. on a mussel farm site) to obtain real time and historical biotoxin, phytoplankton and other environmental data.

Conclusions

- Marine biotoxins are regarded as an important international trade, quality assurance issue in New Zealand
- The public health significance of some “marine biotoxins” is debatable, toxicological research in this area is in progress
- Rigorous shellfish and phytoplankton monitoring programmes permit management to minimise the effects of biotoxin contamination events
- Biotoxins have not seriously affected the economics or viability of aquaculture, there have been no illness from commercial products
- Adoption of new analytical methods will provide a higher level of certainty and security

- Continued research and regular review of procedures and methods are important

Acknowledgements
Various people contributed to this presentation, my thanks to my colleagues at the Cawthron Institute; Paul McNabb, Patrick Holland, Kirsten Todd, Graeme Robertson, Lesley Rhodes, Lorraine MacIntosh, Doug Mountfort, Veronica Buezenberg, and Janet Adamson. Thanks also to Paul Lupi, NZ Mussel Industry Council, Janet Young, NZ MOH, and Phil Busby and Catherine Seamer, MAF Food Assurance Authority.

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Biotoxin Monitoring and Shellfish Farming-Solving Problems in an Essential Partnership

Richie Flynn, Executive Secretary, Irish Shellfish Association

At the first workshop in Cork in 2000, the Irish Shellfish Association (ISA) put forward a number of serious issues that needed addressing - delays in sampling and reporting, lack of species specific closures, lack of plankton monitoring or early warning tools and an absence of any kind of co-ordination. Since then, ISA has been involved at the highest levels on the toxin issue and has succeeded in having many changes implemented into the system. These include the introduction of species specific closures, diethyl ether extraction to rule out false toxins, clear definitions of the roles of the various players, the beginning of plankton testing, the introduction of wide-scale chemical sampling with the aim of replacing the mouse test.

But the industry's main problems remain – vast parts of the most important production areas in the country remain closed or only intermittently open. Producers are still frustrated by unforgivably long periods between sampling and result reporting, resulting in large and quantifiable losses in product recalls and destruction. Mistakes on results have cost producers thousands of pounds. There is a downward spiralling slide in confidence of banks, suppliers, customers and the public with regard to shellfish. The unbelievable financial pressure on farmers and their families is something only the ISA has articulated and which this organisation seems to be alone in caring about. The big question remains – are we being killed by toxins or toxin testing? In creating the most elaborate toxin testing system in Europe, have we built a high-performance machine without a fanbelt or gearstick? Whose foot is on the throttle?

Examples of the basic problems having direct effects on producers are too numerous to mention. The money poured down the drain by being forced to wait 5 days for a return of a sample result has crippled small and large producers alike. Who cares about the man who, having worked for three years to build up his new oyster farm, was forced to recall and destroy his first proud oyster crop because of a late result?

Why are we still seeing eminently solvable problems continuing to cripple producers and where no state agency will take responsibility for compensating for the obvious losses?

- Why are we spending £1 million per annum to monitor a relatively tiny industry?
- Why are we the only country in the world to close bays on the basis of an AZP test?
- Why are we the only country in the EU to close bays at 16 micrograms of Okadaic acid?
- Why are we chasing new toxins that our are continental neighbours are blissfully ignorant of?
- Why do we have an inherently unprofitable industry, where the most efficient and long-established producers have been closed for almost two years?

We are measuring our performance on how fast we can run instead of where we want to go. We have put all the elements in place without adequate co-ordination. We have left the sustainability of the producer behind in our efforts to become the most consumer-protectionist food industry in Europe. Do the alcohol or potato industries have to withdraw products at such low levels of potential toxins?

With all that said, we have established that we have been left with no choice but to work in partnership with the regulators – if not always in harmony. We need to continue to work with the FSAI, the Marine Institute, the Department of Marine and Natural Resources, BIM and the health boards – not for convenience or extra profit, but to ensure that the engine on this bright new machine doesn't completely seize up.

What do we need to achieve in this partnership into the future?

1. Cut out the delays between sampling and results. Appoint a national co-ordinator that is independent with the powers to make changes where they are necessary. The job is simple – get the samples in on time and get the results out on time. If it isn't happening in any bay, get it sorted and keep working until it is sorted. Don't take no for an answer. Let's test the samples over the weekend if necessary.
2. Don't turn shellfish production into a weekend industry. Just because the system works weekdays and turns out a result on Friday afternoon does not mean that the industry has to struggle to fit with that timetable – it must be the other way around. Sample on Friday, result on Monday, and harvest for the week until the next sample goes in. That way we protect the consumer and the producer.
3. Develop plankton sampling into a real early warning system similar to the New Zealand model.
4. Don't allow Ireland to be the EU guinea pig while our competitors race ahead – the playing pitch must be level and no more new tests or systems should be introduced unless they are proven to be in place EU-wide.

We have a quality product in Irish shellfish with an outstanding level of traceability. With a co-ordinated approach among all the players at the same level of partnership seen in New Zealand, we can at the very least establish if it is the toxins in the water or the testing regime which is killing our sector. At the very best we should be able to hold our head high among our peers and sell our fish with the confidence and pride it deserves.

The Ecology and Oceanography of Toxin *Alexandrium* Blooms in the Gulf of Maine: Insights from Model Simulations

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Introduction. The toxic dinoflagellates *Alexandrium fundyense* and *A. tamarense* are responsible for outbreaks of paralytic shellfish poisoning (PSP) throughout the Gulf of Maine in the eastern U.S (Anderson 1997). A 5-year research program called ECOHAB-Gulf of Maine was initiated to characterise the structure, variability and autecology of two major *Alexandrium* habitats in the Gulf using a combination of numerical modeling, hydrographic, chemical, and biological measurements, moored and drifting current measurements, and satellite imagery. This contribution briefly summarises the modelling efforts that have been undertaken, with special emphasis on bloom initiation. One important aspect of *Alexandrium* blooms (Anderson 1998) is that in temperate waters, the inoculum cells arise from germination of dormant cysts in bottom sediments (Fig. 1). Cyst dynamics have thus been an important element of these modelling efforts.

Circulation in the GOM (Fig. 2) tends to be counter-clockwise (Brooks 1985). Superimposed on the south-westward flow of the western GOM are episodic pulses of freshwater from the rivers entering the GOM, producing plumes that extend south-westward along the coast. Of particular importance is the plume called the Western Maine Coastal Current, or WMCC. Previous to this study, it was known that blooms of *Alexandrium* in the western Gulf are closely associated with the WMCC (Franks and Anderson 1992).

Modelling.
Early numerical modelling work has demonstrated the importance of these plumes in the transport and fate of the cells and the dynamics of PSP toxicity (see http://woodshole.er.usgs.gov/operations/modeling/wgulf/modeling.html.) In particular, these model runs demonstrate how toxicity can fluctuate with different wind forcings. For example, when the wind blows from the south-west, the plume is pushed rapidly offshore in a thin layer only a few meters thick. Nearshore waters become colder as deep, upwelled water comes to the surface. In this instance, the nearshore shellfish would not become toxic, or would decrease in toxicity if they were already toxic, since the plume and its associated *Alexandrium* cells would be carried away from shore due to the upwelling-favorable winds (Fig. 3, top panels). A wind event from the north-east would induce downwelling, which pushes the plume water shoreward and delivers the cells to the coast (Fig. 3, bottom panels).

For these initial simulations, *Alexandrium* vegetative cells were arbitrarily introduced into the model at a constant rate at a location near the mouth of the estuarine system that produces the WMCC water. The next step in model development was to use a mapped cyst seed-bed (Fig. 3) to provide a more realistic inoculum. *Alexandrium* cysts were found throughout the region, but peak concentrations were seen in a large seedbed, located 30-50 km offshore

and seaward of the 100m isobath (Fig. 4). Despite the high cyst abundance offshore, it was thought that cysts in shallow coastal embayments would germinate quickly as daylength increased and waters warmed in the spring. This would supply a large, synchronous pulse of cells that could be responsible for an inshore initiation of the bloom. Offshore cysts in deeper waters would germinate slowly due to the cold and dark conditions, and those cells that did germinate would be too far offshore to participate in the coastal blooms.

A number of experiments were conducted in the laboratory using cysts in natural sediments in order to parameterise their germination behaviour in response to variations in light, temperature, and other factors. When the mapped cyst distribution was used in the model, it was demonstrated that cells germinating from cyst seedbeds in the deep, offshore waters could swim up into the plume when it was blown offshore by upwelling-favorable winds. A subsequent downwelling episode could then carry these cells back to shore with the plume, where they could grow and cause toxicity in nearshore shellfish. This pattern of offshore bloom initiation was observed in survey cruises in 1998 and 2000. When this mechanism was explained to Maine officials, they modified their shellfish monitoring program by establishing shellfish toxicity testing sites at offshore islands that could provide an "early warning" for toxin development.

The model domain is now being extended to the east so that it includes the entire Gulf of Maine region. Efforts are also underway to conduct sensitivity studies to initial cyst distributions, excystment rates, and growth processes in an attempt to isolate key factors that currently limit our ability to simulate observed cell distributions. In the future, the understanding that stems from projects like ECOHAB-GOM will enable us to construct realistic simulation models driven by real time data collected at strategic locations to predict the landfall of red tide blooms in the Gulf of Maine.

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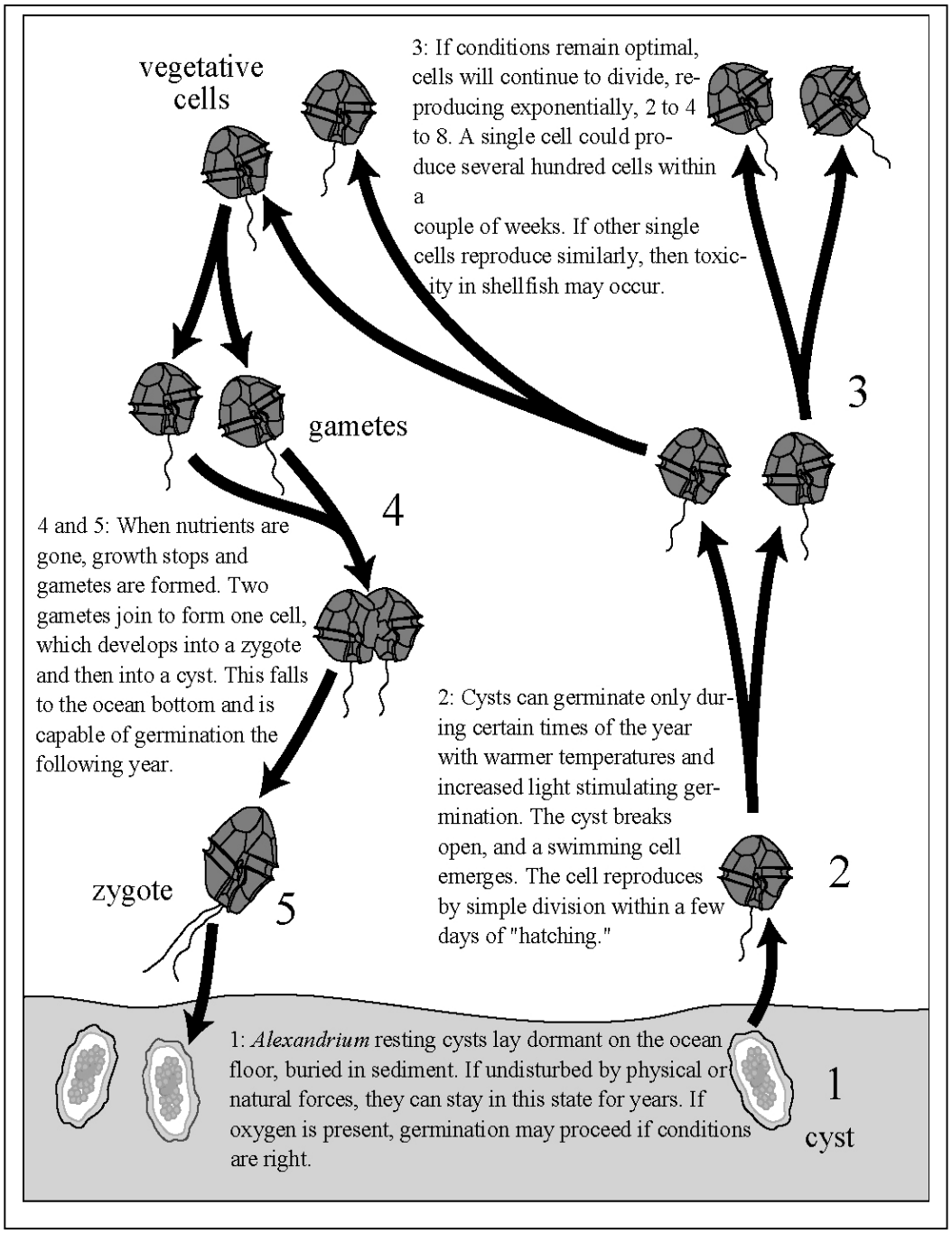

Figure 1. The life cycle of an *Alexandrium sp.* cell.

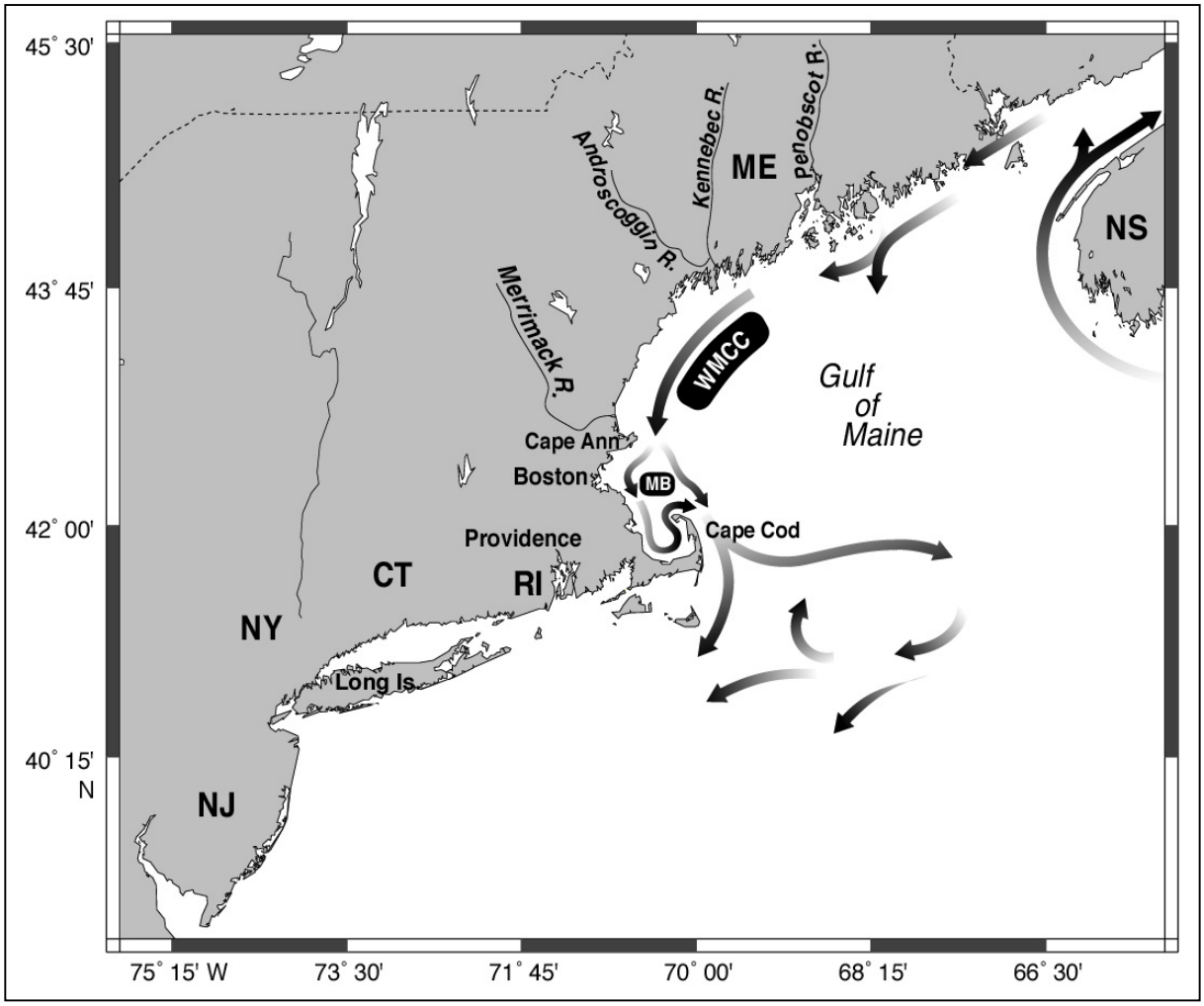

Figure 2. *Alexandrium* subpopulations or habitats in the north-eastern U.S. Six regional populations (black boxes) are identified, defined by circulation patterns and the discontinuous distribution of the dinoflagellate: BOF – Bay of Fundy; EMCC – eastern Maine coastal current; WMCC – western Maine coastal current; MB – Massachusetts Bay (includes Cape Cod Bay); Georges Bank; and southern salt ponds and embayments. Qualitative surface circulation patterns are indicated by arrows.

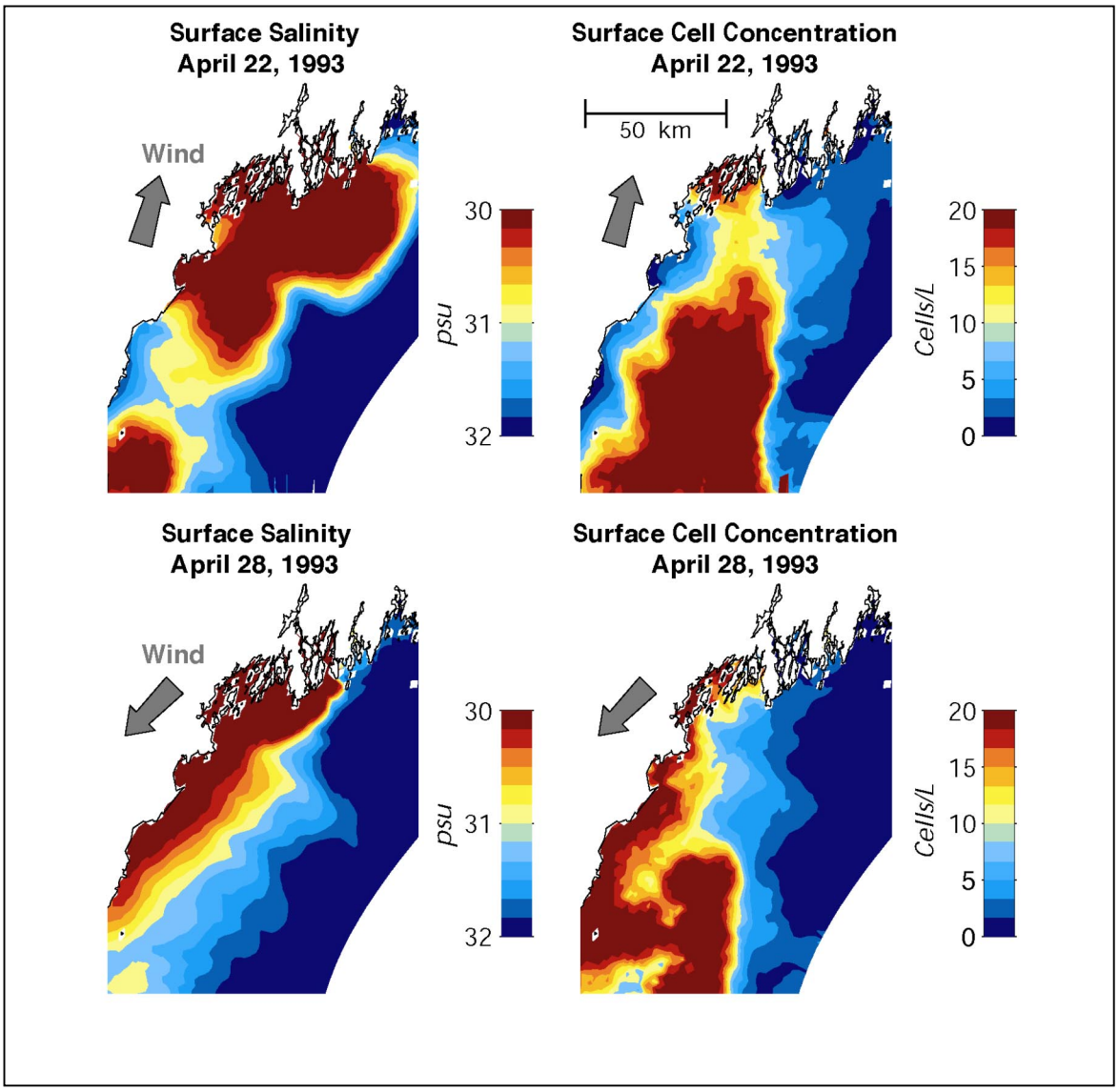

Figure 3. Model results showing surface salinity and *Alexandrium* cell concentrations in the Gulf of Maine in April 1993. The top panels show the effect of upwelling-favorable winds, which move the *Alexandrium* cells offshore, leaving low cell concentrations near shore. The bottom panels depict downwelling-favourable winds, which push the buoyant plume and its *Alexandrium* cells back to shore, with rapid propagation of toxicity alongshore.

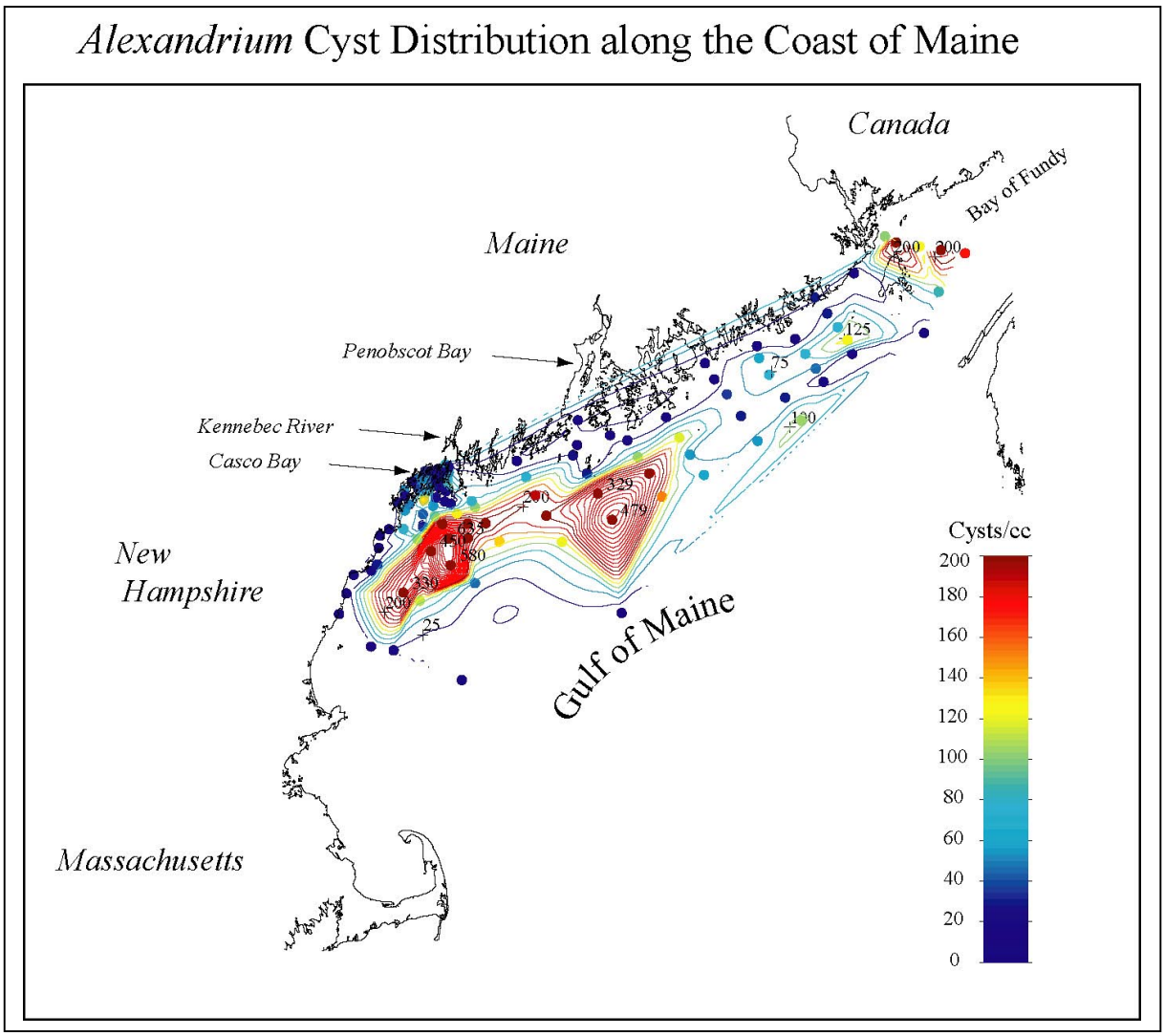

Figure 4. Distribution of *Alexandrium* cysts (cm^{-3}) in surface sediments of offshore coastal waters of the Gulf of Maine in 1998. Values are for the top 2 cm

Update on AZP and ASP research at Cork Institute of Technology and University College Cork

Dr. Kevin James, Cork Institute of Technology Ecotoxicology Unit.

Summary of Paper delivered by Dr. Kevin James was not available for inclusion at time of publication.

Concluding Discussion

Dr. Patrick Wall, Chief Executive, Food Safety Authority of Ireland

Despite much recent progress the biotoxin issue remains a source of frustration to producers, processors and the regulatory agencies. Regulatory approaches should be practical, equitable compliant with EU requirements and proportional to the risks. The objectives are to protect consumers' health and the reputation of the Irish shellfish industry. The conference has highlighted many of the issues pertaining to phytoplankton and biotoxins and illustrated that Ireland is not alone in having to cope with the problem.

Sampling

The efficiency and effectiveness of the National marine biotoxin monitoring programme depends firstly on a good system of sample collection and timely delivery to the laboratory for analysis. Samples must be properly labelled and of adequate size. To assist this process all of the bays around the coast of Ireland have been mapped and sampling points identified in each production area. The compilation of biotoxin sampling codes for all sample sites is now complete and these codes are to be introduced from the 1st of November 2001. By January 2002 all codes must be fully in use and the labs will not accept miscoded, poorly coded or wrongly coded samples after that date. Sampling is the responsibility of the Sea Fisheries Officers and additional Officers have been employed to ensure that the system operates effectively. The Department of Marine and Natural resources will circulate pre-printed coded labels to all Sea Fisheries Officers (Shellfish Managers).

Communication of Results

A sub group of the Molluscan Shellfish Safety Committee was developed to investigate ways of speeding up the process of reporting results. The time lag is still unsatisfactory in terms of feedback. The production of a single homogenate for both mouse bioassay and chemical analysis would cut out some of the duplication that is currently in the system and would increase speed and accuracy of results. It is also necessary to prioritise open bays where harvesting is ongoing.

AZA Toxicology Report – Emiko Ito, Terry Mc Mahon et al

This paper is to be published in 'Toxicon' in the near future. Further research will be necessary to support the findings outlined and the response needs to be proportionate. The FSAI Scientific Committee is reviewing the paper from a food safety perspective and they will advise in December 2001.
The issue of bioavailability of AZA was raised and it was agreed that further research is required in this area.

AZA Toxicology Working Group

A working group has been established to arrange to have further toxicological work carried out on AZA. However, at present there is no toxic material available to undertake the research proposal submitted by Prof. Michael Ryan. The working group is to meet again to examine alternatives and to determine whether it is possible, in the absence of toxic material, to proceed.

The Marine Institute is currently working on isolation of AZA toxin, but this will take several months.

Codes of Practice
The working groups are to meet to finalise the 'guidance note on the Control of Marine Biotoxins in Harvesting and Processing of Bivalve Molluscs' and the 'Code of Practice for the Monitoring of Marine Biotoxins in Bivalve Molluscs'. A decision tree is to be included in the guidance note to indicate what action is required when stock is found to be positive and the steps to be taken in the case of product recalls. The issue of when harvesting is allowed is to be clarified.

Chemical Testing
Contract negotiations have now been completed and the Marine Institute is awaiting a decision from the Department of Marine and Natural Resources. The Marine Institute are doing multi toxicity testing whereby the same homogenate will produce a result for okadaic acid and azaspiracid. They hope that this will form part of any new arrangements put in place. There is currently some duplication of analysis between the Marine Institute and CIT.

Information being generated should be used in full to inform decisions and the Marine Institute is to carry out analysis of data on a quarterly basis. Useful analysis would include an examination of trends, anomalies in the system, toxin profiles in production areas, predictive models etc. Chemical analysis must continue to allow useful information on toxin profiles of bays at different times of the year to be developed. However, the use of the okadaic acid chemical test coupled with the mouse bioassay as a means of detecting DSP to be re-examined as one test should be sufficient. The chemical test for okadaic acid may be continued as a research facility and high levels of okadaic acid may be used by industry as a means of implementing voluntary closures.

Industry representatives accept the need for data collection but they want the decision on opening and closing of bays with respect to DSP to be made on one test or the other, not on both the mouse bioassay and the Okadaic acid chemical test. The Marine Institute demonstrated that there was a 93% correlation between the results of the chemical testing and the mouse bioassay. It was agreed that in the 5% of cases where there was a contradiction a review of these results would be undertaken.

Harmonisation of Testing Regimes – Draft EU Decisions
The Standing Veterinary Committee (SVC) passed both decisions on Marine Biotoxins at the end of October 01. They will inform Member States as to the acceptable limits in the case of all marine biotoxins and they also set out the methods that are acceptable for the detection of each marine biotoxin. All Member States must accept the use of methods outlined in the decisions.

Classification of Bays
A number of Bays went from A classification to B classification in the new designation order signed by the Minister on the 7th of August 2001. The

Department of Marine and Natural Resources informed the ISA of this on the 13th of August 01. The ISA have written to the Minister asking for the problems of pollution in Shellfish producing areas to be addressed and for all bays to be designated under S.I. 200 of 1994 (Quality of Shellfish Waters Regulations 1994). Where designated bays were downgraded the sampling frequency has been increased to weekly sampling and reclassification will take place again in November of 2001. The quality of the water in which shellfish are grown is an extremely important factor in ensuring the product is safe for human consumption and proper sewage treatment and management of agricultural effluent are essential to ensure bays are not being polluted.

Phytoplankton

The Marine Institute continues to build up a databank of information on phytoplankton in the shellfish production areas. New methods of testing and analysis are being investigated. Results of analysis are sent to the producers and the intention is to develop early warning system. Useful information is being gathered and should be taken in account when making future decisions.

Conclusion

Progress has been, and is being, made but much remains to be done. Producers, processors, the Department of Marine and Natural Resources, the Marine Institute, the other laboratories and the Food Safety Authority of Ireland share a common vision to see a vibrant industry producing a safe product. Using the creative talent of all the stakeholders we will recognise, define and solve the problems we are encountering leading to improvements in the monitoring and regulatory systems and to the placing on the market of a safe product of consistent high quality.

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