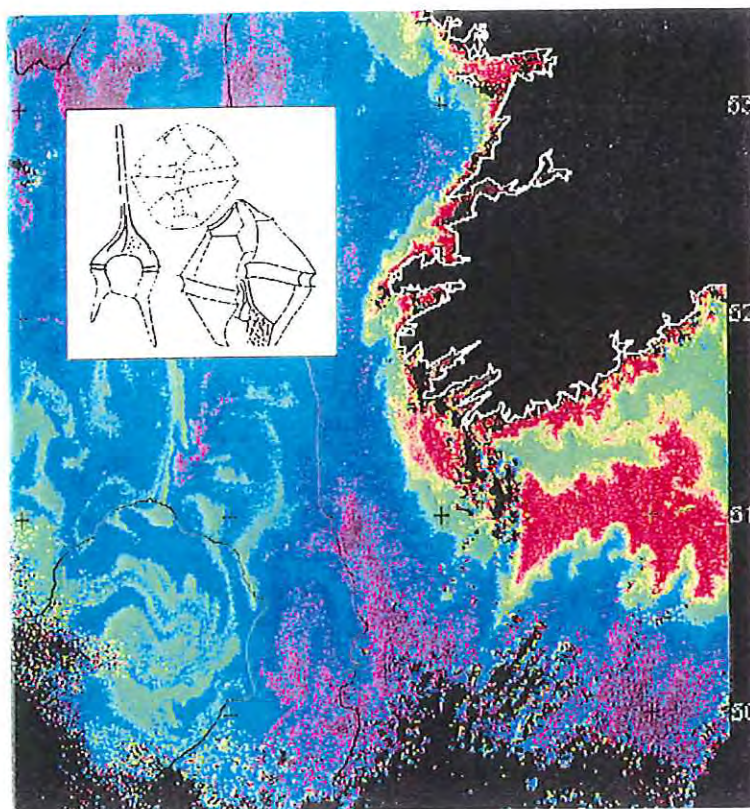




Marine Institute
Foras na Mara

Proceedings of the First Irish Marine Biotoxin Science Workshop



Cork 19th April, 2000



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Agenda

19th April, 2000

9.00am Registration

10.00-10.10 Chairman: Pat Keogh, Chief Executive, Bord Iascaigh Mhara

10.10-11.0 Review of the Current Irish National Biotoxin Monitoring Programme

Methods, Agencies, Costs, Strengths And Weaknesses.

Michael O'Driscoll, Sea Fisheries,

Department of the Marine & Natural Resources.

Micheal O'Cinneide, Marine Institute

Dr. Terry McMahon, Marine Institute

11.00-11.15 Tea/Coffee

11.15-11.45 Industry Biotoxin Management Programme In Ireland

Methods, Agencies, Costs, Strengths and Weaknesses.

Liz Abbott, Quality Manager, Bantry Bay Mussels Ltd.

Richie Flynn, Executive Secretary, Irish Shellfish Association

11.45-12.30 Azaspiracids Research

Dr. Terry McMahon, Marine Institute

Dr. Kevin James, Cork Institute of Technology

Andrew Flanagan, Bioresearch Ireland

12.30-1.0 Discussion

Chairman: Dr. Gavin Burnell, National University of Ireland, Cork

2.30-3.0 Overview of Biotoxin Monitoring regime in New Zealand.

Responsibility and Funding

Dr. Terence O'Carroll, Bord Iascaigh Mhara

Joe Silke, Marine Institute.

3.00-3.15 Overview of Biotoxin in Scotland.

Godfrey Howard, Shellfish Hygiene Group, Fisheries Research Services,
Marine Laboratory, Aberdeen.

3.15-3.30 Tea/Coffee

3.30-4.0 Phytoplankton-Research and Monitoring Priorities

Dr. Robin Raine, National University of Ireland, Galway
Joe Silke, Marine Institute.

4.00-4.30 ASP Toxin

Dr. Kevin James, Cork Institute of Technology
Dr. Susan Gallacher, Fisheries Research Services,
Marine Laboratory, Aberdeen.

4.30-5.0 Biotoxin Monitoring Regime in Ireland-2000 and Beyond

Micheal O' Cinneide, Marine Institute
Dr. Catherine Butler, Bord Iascaigh Mhara

5.00-5.30 Discussion

Extract from Sea State, Issue 9, Summer 2000 First Biotoxin Workshop in Cork

"The Marine Institute joined forces with BIM and the Irish Shellfish Association to host the first national Marine Science workshop on Biotoxin management in Cork on 19 April, 2000.

Over 70 people attended the workshop, which included a cross section of Irish shellfish producers, researchers and state agencies. The event was modelled on the Marine Science Biotoxin Workshop, which has operated successfully in New Zealand for the past 5 years.

As Micheal O Cinneide, Divisional Manager in the Marine Environment & Health Services Division said, **"The aim of the seminar is to promote dialogue between industry, scientists and state agencies on effective monitoring and safer shellfish"**.

The distinguished line up of speakers included Dr. Kevin James and Dr. Ambrose Furey from Cork Institute of Technology; Dr. Susan Gallagher and Godfrey Howard from the Marine Lab in Aberdeen; Dr. Robin Raine of NUI Galway; Andrew Flanagan from BioResearch Ireland; Dr. Terry McMahon and Joe Silke from the Marine Institute; Pat Keogh and Dr. Catherine Butler from BIM and Michael O Driscoll from the Department of Marine; Richie Flynn from the Irish Shellfish Association.

Some of the key messages which emerged from the Seminar were:

- Ireland spends £500,000 per year on biotoxin monitoring and research
- The development of a reliable test method for a new toxin, Azaspiracid, is a top priority for Irish researchers
- Ireland has an effective biotoxin monitoring regime, but closer coordination is needed to achieve our aim of being a leader in biotoxin management in the Northern hemisphere!"

Opening Remarks at Marine Science Biotoxin Workshop
Pat Keogh, Chief Executive, Bord Iascaigh Mhara

- Welcome to all present, thank all for making the journey to Cork. We have people here from right around the coast and as far afield as Scotland. I am glad to see many familiar faces and some new ones.
- This is the first workshop of its kind to be held in Ireland. The idea for it came from the recent trip to New Zealand and Tasmania by members of the industry, the Marine Institute and BIM. The workshop is jointly organized by the Marine Institute and BIM with support from the Department of the Marine and Natural Resources, and the Irish Shellfish Association.
- The idea behind the workshop, learning from the New Zealand experience where such workshops are held bi-annually, is that it be an open and constructive discussion forum. It is not a policy-making session but more a chance for all interested parties to catch up on the latest developments in the field of biotoxin study and also to critically examine our own biotoxin monitoring system in the light of experience gained in other countries as well as our own experience.
- The situation with biotoxins is constantly changing. It requires vigilance and a process of updating for the industry and the state to keep pace with this naturally occurring natural phenomenon. I am particularly pleased to see a high level representation from the Food Safety Authority of Ireland here today as I believe it is very important for those concerned with food safety to have the fullest possible understanding of the biotoxin phenomenon.
- Some years ago at the behest of the then Minister of State at the Department of the Marine, I chaired a Biotoxin Review Group which produced a report. I am pleased to say that much of what was recommended there was subsequently implemented. However, even in the intervening period we have had new challenges, not least the recent emergence of the *azospiracid* toxin which shows that we must constantly work to improve and update our systems and procedures.
- At BIM our primary concern is for the further development of the shellfish industry which has enormous potential benefit for our coastal communities. Such development can only take place against a backdrop of quality assurance and food safety. I know from my own personal experience that the industry, the Department and State agencies are committed to the highest levels of food safety and I earnestly hope that today's workshop will contribute meaningfully to the debate and to continue the progress that has already been made in the area.



Marine Institute / BIM / Irish Shellfish Association

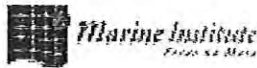
First Irish Marine Biotoxin Science Workshop

Cork, 19 April, 2000

*Aim: To promote dialogue between industry,
scientists & State agencies
on effective monitoring & safer shellfish*

Review of Irish Biotoxin Monitoring Programme

Micheal Ó Cinnéide and Terry McMahon,
Marine Environment & Health Services,



Irish Monitoring Programme

- Managed by Department of Marine,
under a Service Contract with FSAI
- Plan based on EU Directive 91/492
- Marine Institute is the National
Reference Laboratory for Biotoxins
- Results compiled by Marine Institute
and faxed weekly to over 130 contacts

DSP Monitoring Programme

- ┆ Programme commenced in 1984
- ┆ Phytoplankton species - *Dinophysis acuta* and *Dinophysis acuminata*
- ┆ 967 samples tested in 1998
- ┆ 1,850 samples tested in 1999
- ┆ Regional DSP labs in Cork and Mayo
- ┆ 24 hour Mouse Bioassay, as in France, Spain, Portugal, Belgium, Denmark & Austria

PSP Monitoring Programme

- ┆ PSP - Detected above limits in Cork Harbour mussels in 1996, 1997 and 1998
- ┆ Phytoplankton species - *Alexandrium tamarense*
- ┆ Marine Institute has identified cysts of *Alexandrium tamarense* in sediments in Cork Harbour

ASP Monitoring Programme

- ┌ ASP - Detected above limits in scallops from Mulroy, Connemara, Valentia and Sneem in December 1999 and other locations - SE and East coast in 2000
- ┌ Toxin - Domoic acid
- ┌ Phytoplankton species - *Pseudo-nitzschia*
- ┌ Cork IT and Marine Institute monitor using the HPLC chemical method

Azaspiracid Monitoring Programme

- ┌ Azaspiracid - First detected in mussels from Killybeg in November 1995
- ┌ Phytoplankton species - Unknown to date?
- ┌ Monitoring method - Mouse Bioassay
- ┌ Cell test under development
- ┌ Research underway in Cork IT and BioResearch Ireland

Costs of Irish Biotoxin Monitoring & Research Programme

- ┌ Marine Institute - £250,000+ in 2,000
 - ┌ Marine Research measure - £50,000 pa on new Azaspiracid test method in 99/2000
- ┌ BIM Grant and Direct costs - £100,000 pa on Industry related monitoring.
- ┌ Cork IT - £75,000 pa on Biotoxin research
- ┌ Public Analysts Labs - £25,000+ pa
- Total State investment - **£500,000**

Value of Irish Molluscan Shellfish Exports, 1998

- ┌ Mussels - £25 million
- ┌ Oysters - £ 4 million
- ┌ Scallops - £ 2 million
- ┌ Total Molluscs - £31 million
- ┌ Biotoxin costs = 1.5% of export sales

Strengths of Irish Biotoxin Monitoring programme

- Scientific Expertise in Marine Institute, and in Cork Institute of Technology
- Favourable results on DSP and ASP in EU and Irl/UK Inter-Calibration tests
- Strong DOM/MI/BIM/ISA linkages
- In 1999 over 80% of harvesting areas were tested

Challenges for of Irish Biotoxin Monitoring programme

- Complex toxin profile in Irish waters
- Build knowledge re Azaspiracids + ASP
- Improve regularity in Sampling
- Achieve test coverage in all Molluscs
- Develop automated, in situ monitoring
- Develop reliable new predictive tools

Molluscan Safety Committee

- ┌ Represents DOMNR/BIM/MI/Industry
- ┌ Provides detailed management and co-ordination of the operation of the National Biotoxin Monitoring Programme
- ┌ Facilitates inter-agency and inter-departmental co-operation in the logistics of the programme

Molluscan Safety Committee

- ┌ 1999/2000 focus on sampling and sampling logistics
- ┌ Sample locations, sample frequency, species, sample delivery to labs
- ┌ Revised National Sampling plan to be circulated in May 2000
- ┌ Regional meetings and training workshops

Update on EU Biotoxin Meetings

- ... Ireland attends annual meetings of Network of National Reference Laboratories held at EU Reference Laboratory in Vigo, Spain
- ... Expert Working Groups / Panels set up in 1999 - ASP, PSP Mouse Bioassay, DSP toxins, Toxicology, Protein Phosphatase assay (DSP), Mouse Neuroblastoma assay (PSP)

EU Meeting 2000 - Azaspiracid

- ... Recommendation- "Azaspiracid should be monitored in all Member States"
- ... Recommendation - "Azaspiracid should not be included in DSP toxin complex"
- ... Amendment of Directive 91/492 needed
- ... Expert Panel on Toxicology will examine available data on azaspiracid and if possible set a human health standard

EU Meetings - ASP in Scallops

- ┆ Analyse total tissue - if $< 20 \mu\text{g/g}$, harvest allowed for fresh and processed product
- ┆ If $> 20 \mu\text{g/g}$, restricted harvesting
- ┆ Test meat and gonad - if $< 20 \mu\text{g/g}$ product can be sold
- ┆ If $> 20 \mu\text{g/g}$, material must be destroyed

EU- Inter Laboratory Studies

- ┆ Inter-laboratory study to validate ASP analysis, using the Quilliam method - late 2000/early 2001
- ┆ Inter-laboratory study on equivalency of methods currently used in EU for DSP determination - October 2000
- ┆ Inter-laboratory study on Yasumoto (1978 and 1984) DSP mouse bioassay with standard protocol and whole body assay.

Biotoxin Regime The Industry View



Richie Flynn
Executive Secretary
Irish Shellfish Association

Industry Participation



1995 Task Force
Liaison Group
Molluscan Safety Committee
Sampling effort
Feedback to Customers

Industry Objectives



Safe Shellfish

Current system protects consumers and regulators but does not protect producers

e.g. Early Warning System - costs

A regime with both consumer and producer confidence

First concerns



Autumn 1998

Change from 5 hr to 24 hr

Rapid Health Alert

Erosion in communication had led to reduced sampling effort

Changes in sampling areas

Long periods of closure

Amplified Concerns



- Over-long period required for re-opening (MI)
- Species Specific Closures (MI)
- Adequate Testing capacity (MI)
- Improved communication (MI)
- Badly handled PR (Dept/health boards)
- Co-ordination of sampling effort (Dept/BIM/MI)
- "Out of Character" results (MI)

Amplified Concerns



- New Toxins Research (MI)
- Harmonisation of regime with other member states (MI/BIM)
- Predictive modelling - learning from NZ experience (MI)
- Much improved water pollution protection in line with 79/923 EC (Dept)
- New Gatherers Document (Dept)

Action on industry concerns



MI / BIM actions completed

- Adequate Testing capacity (MI)
- Improved communication (MI)
- Harmonisation of regime with other member states (MI/BIM)
- Predictive modelling - learning from NZ experience (MI/BIM)

Actions in train



Over-long period required for re-opening (MI/MSC)

Species Specific Closures (MI/MSC)

"Out of Character" results (MI/MSC)

New Toxins Research (MI/Research Centres)

Outstanding issues



Much improved water pollution protection in line with 79/923 EC (Dept)

New Gatherers Document (Dept)

Badly handled PR (Dept/health boards)

Co-ordination of sampling effort (Dept/BIM/MI)

The Future



Producer Onus

If all responsibility for testing/ recall/ destruction lies with producer then the State cannot walk away from its responsibilities under 79/923 EC

Industry & general public want cleaner inshore waters not closures and de-designations.

The Future



Before the issue of sharing costs (beyond the current significant costs to industry in gathering and dispatching samples and beyond what is expected of other EU industries) is broached at all outstanding issues must be resolved

particularly co-ordinated sampling, PR, reduced opening period, Species Specific Closures and proper implementation of 79/923

No question of industry getting involved in costs to bring current system up to standard

Azaspiracid Research

Dr. Terry McMahon

Azaspiracid

- November 1995 report of illness in Holland following the consumption of mussels from Killary Harbour
- Symptoms - Severe diarrhoea, vomiting, nausea, stomach cramps, headaches and chills persisting for 3 - 5 days
- Positive DSP rat bioassay results
- Positive DSP mouse bioassay results
- No pathogenic bacteria detected
- No known toxins detected chemically
- No toxic phytoplankton detected



Azaspiracid and its analogs

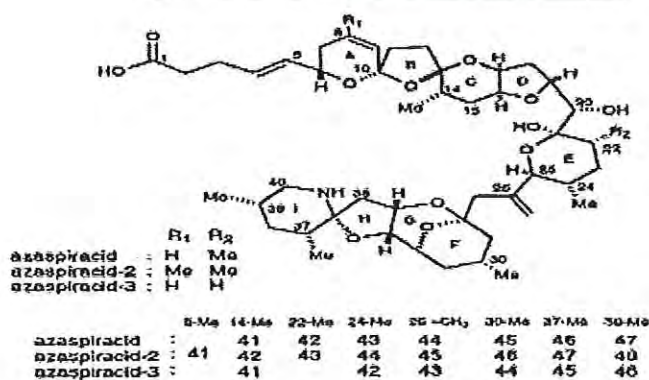


Figure 1. Structures and carbon numbers of methyls and exomethylene of azaspiracid and its analogs.

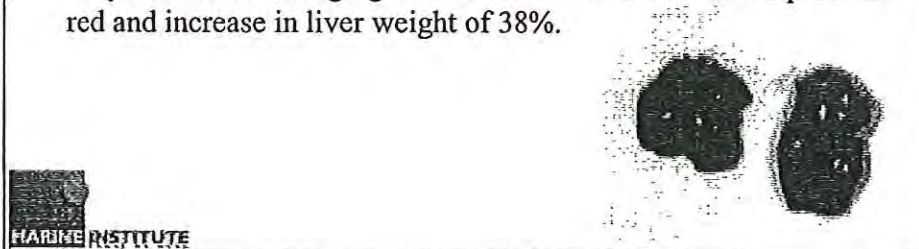


Azspiracid in Irish Shellfish



Azspiracid

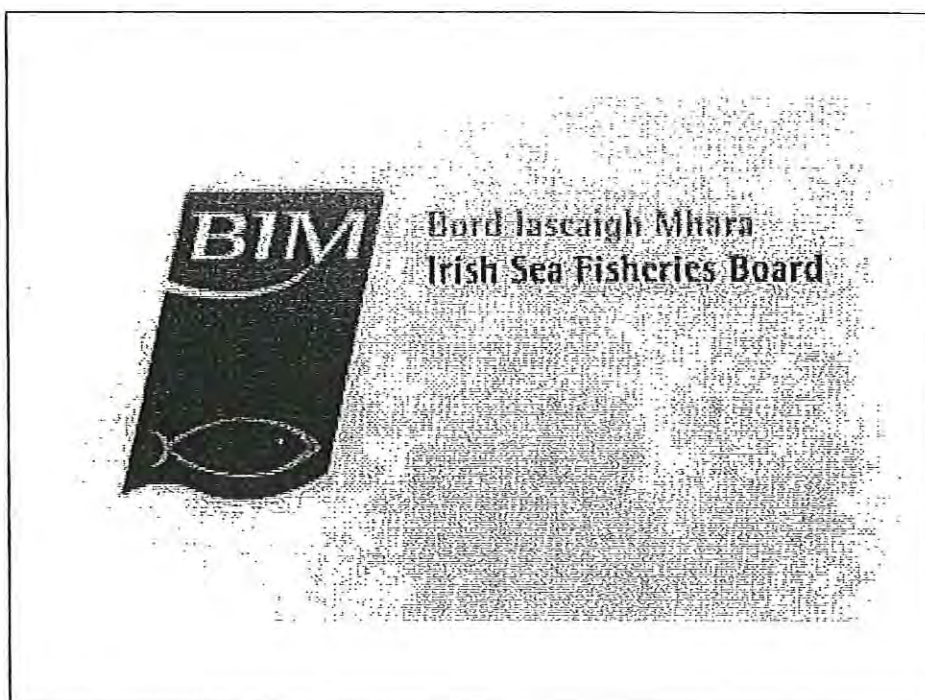
- **Human illness** - Vomiting, nausea, diarrhoea, stomach cramps, headaches and chills, lasting typically 3 -5 days
- **I.P injection of acetone extracts** - respiratory difficulties, spasms, paralysis of hind legs, death within 30 - 90 minutes at high doses. At lower doses death may occur within 24 hours.
- **Oral administration of purified azaspiracid**- fluid accumulation and necroses of villi in small intestine, necroses in thymus and spleen, damage to T and B lymphocytes, accumulation of fat droplets in liver changing colour of liver from dark red to pinkish red and increase in liver weight of 38%.



Azaspiracid

- The source of the toxin is still unknown
- The exact mode of action is unknown
- A robust analytical method is not yet available
- A safe level for human consumption has not yet been determined
- Purified toxin standards and standard reference material are required for analytical purposes, development of alternative assays e.g cytotoxicity, ELISA, toxicology
- Currently the best available monitoring method is the DSP mouse bioassay (Yasumoto, 1978) with a 24 hour threshold.





Biotoxin Monitoring In New Zealand
And
Its Relevance To The
Irish Shellfish Industry.
Dr. Terrence O'Carroll, BIM

Rope Mussel Industry

New Zealand Green Lipped Mussel *Perna canaliculus*.

In 1979 production 1000 t.

In 1999 production 60,000 t.

Final processed value over £62 million

Over 10% of total seafood production and value.

90% of production processed by 5 main processors.

History of Monitoring Programme

Set up after Memo of Understanding with USA - FDA in 1980.

Since early 80's National policy of user pays.

Export worldwide - meet FDA and EU criteria.

MAF overall responsibility, Ministry of Health has national remit.

No real problems until 1993!!

In 1994 15,000 samples tested .

In 1999 5,200 tested.

Industry decided if they are paying they should have more input.

Started Marine Biotoxin Science Workshops.

Industry and Regulators agreed standards and monitoring programmes.

Monitoring Programme

Programmes carried out by industry on a regional basis.

Marlborough Sound Shellfish Quality Programme (MSQP).

Commercial scallops have separate Southern Scallop Marine Biotoxin Management Plan.

Industry pays so industry awards contracts.

MAF still overall responsibility to monitor programme.

Nationally there are 70 phytoplankton samples a week - have associated trigger levels.

MSQP - 16 sites tested on weekly basis for flesh and phytoplankton with an additional 8 sites tested for phytoplankton.

All samples collected by Sampling Officers under the control of the Senior Health Protection Officer.

HPO/AHO have power to authorise whatever additional samples or testing required.

NSP - Wait for phytoplankton trigger.

DSP - Weekly on flesh.

PSP - 2 weeks or 4 weeks depending on area.

ASP - 2 weeks or 4 weeks depending on area.

MAF monitor monitors!

Closure notices issued by HPO - includes harvest area and buffer zone for public health.

MAF impose ban on harvesting areas.

MAF ensure export consignments non toxic.

HPO contacts non MAF registered dealers.

Costs

MSQP = £375,000 (NZ\$900,000)

• Bioassay testing	\$300,000
• Admin and Communication	\$200,000
• Phytoplankton	\$150,000
• Sample collection	\$100,000
• Micro analysis	\$100,000
• Contribution to MAF	\$50,000

Industry also funds and supports research.

Overview of Biotoxin Monitoring Regime in New Zealand
Joe Silke



3/2/01

Biotoxin Monitoring in New Zealand

Irish Marine Biotoxin Science Workshop

19th April 2000

by

Joe Silke

Marine Biotoxin Reference Lab

Marine Institute

Abbotstown

Dublin 15

Biotoxin Monitoring in New Zealand

- ✦ **Screen Tests.**
- ✦ **Phytoplankton as a screening method.**
- ✦ **rRNA (Gene) Probes.**
- ✦ **Integration of gene probes into phytoplankton monitoring programme.**
- ✦ **MIST Alert™.**

Screen Tests

- ✦ **99.5% of New Zealand shellfish tests are negative**
- ✦ **Screening tests to identify toxic shellfish flesh approaching the MPL, to reduce animal tests and may be quicker.**
- ✦ **Using these screen tests, negative shellfish could be harvested, while positive shellfish would be re-tested using bioassay**

Screen Tests

- † ELISA is selected as the proposed analytical technique for development, quick, single extraction for all toxins, using no animals.
- † Depends upon the availability of antibodies with sufficiently broad cross-reactivities to ensure a toxic event caused by any member of the toxin group will be detected.

Screen Tests

Ireland:

- † The concept of a screening test is desirable to eliminate negative tests
- † Whether this is possible with the use of ELISA tests, cytotoxicity assays, bio-chemical methods or others has yet to be established.
- † DSP/AZP Cytotoxicity Assay and PSP Lateral Flow test are to be lab tested by Marine Institute in the coming months as potential replacement / screen tests.

Phytoplankton Screening

† Comprehensive phytoplankton monitoring programme in place

† Concentrate on areas of Green Lipped Mussel cultivation



Phytoplankton Screening

† 22 species of harmful phytoplankton toxic via shellfish or aerosols, water discolourants or fish killing.

† As part of the biotoxin programme, cell counts are used to trigger flesh tests, as it is thought that this is quicker, cheaper than flesh testing, and the product can be left to naturally depurate.

Phytoplankton Screening

Ireland:

- ✦ Similar lab methods: Utermöhl sedimentation and Calcifluor thecal plate fluorescence methods.
- ✦ In addition they have started to use rRNA targeted probes for the discrimination of toxic *Pseudonitzschia* and *Alexandrium* species. These methods are being tested in the next month in Dublin.

Phytoplankton Screening

Ireland:

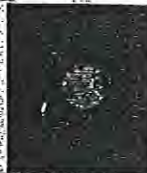
- ✦ The phytoplankton trigger levels for flesh testing are similar to that which is currently operated in Ireland for *Alexandrium* and PSP monitoring.
- ✦ However the occurrence of DSP in New Zealand is different to our (and the general NW European) experience of the toxin

rRNA (Gene) Probes

† A means to distinguish morphologically similar species and strains

† In all cases these probes serve to reveal either a particular species or a group of species, specifically,

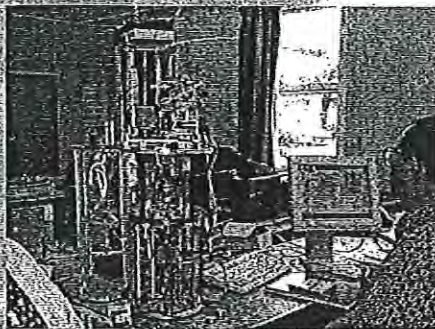
† rRNA Probes are currently developed to distinguish a range of HAB species.



rRNA (Gene) Probes

† An example of where this technology is heading may be forecasted by the development of this phytoplankton analysis Robot

† This is designed to be moored at sea where it will automatically sample, analyse, photograph and report back to land the occurrence of harmful species



rRNA (Gene) Probes

Ireland:

† The Irish monitoring of HAB species is severely hampered by the lack of tests to identify and quantify morphologically similar species that occur in natural assemblages.

† A study of this technology is about to commence in the Marine Institute, in an initial attempt to adopt the technique and assess it's effectiveness in the Irish context.

Integration of probes into Monitoring

† Whole cell probes are used routinely for the discrimination of toxic from non-toxic *Pseudonitzschia* sp. and have routine use in resolving *Alexandrium catenella* from the non toxic *A. fraterculus*.

† Detection of *Pseudonitzschia* RNA in seawater following the collapse of a bloom.

† Detection of *Heterosigma akashiwo* in field samples.

Integration of probes into Monitoring

Ireland:

- † Possibility of using SHA for the detection of ichthyotoxic phytoplankton such as *Heterosigma* and *Gymnodinium* species.
- † Both of these have caused extensive salmon mortalities in the past and often the bloom has crashed before water samples are available for identification of the responsible species.
- † May be useful in resolving toxic and non-toxic strains of *Alexandrium tamarense* in Irish waters.

'MIST Alert'™ PSP Test Kit

- † The test is a lateral flow format competitive enzyme linked assay with a colourimetric detection similar to the common home pregnancy test format.
- † Test is due for shipping for next year following lab testing.
- † The new MIST Alert tests are qualitative (yes/no) tests

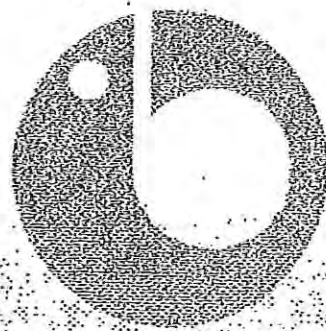


Summary

- † A number of new products and methodologies are being developed to facilitate more effective biotoxin monitoring
- † A NZ predictive management system which is the ultimate aim is thought to be within five years from becoming a reality
- † Some of these new technologies are appropriate to the Irish situation, and currently several new techniques are being investigated.

MARINE BIOTOXINS

MONITORING, SURVEILLANCE AND CONTROLS IN THE UK



**Marine
Laboratory
Aberdeen**

LEGISLATION

1. THE SHELLFISH HYGIENE DIRECTIVE 91/492/EEC
2. THE FOOD SAFETY (FISHERY PRODUCTS AND LIVE SHELLFISH) (HYGIENE) REGULATIONS 1998

A STATUTORY REQUIREMENT UNDER :

A.

- a) Shellfish Hygiene Directive : 91/492/EEC
- b) The Food Safety (Fishery Products and Live Shellfish) (Hygiene) Regulations 1998 (S.I. 1998 No.994)

THIS LEGISLATION REQUIRES THAT MONITORING IS UNDERTAKEN FOR :

1. MARINE BIOTOXINS
 - a) Monitoring of harmful phytoplankton
 - b) Monitoring of marine biotoxins in shellfish flesh
2. MICROBIOLOGICAL CONTAMINATION
 - Monitoring of *E.coli* levels in shellfish flesh for harvesting area classification
3. CHEMICAL CONTAMINANTS
 - Monitoring of chemical contaminants in shellfish flesh

This work is undertaken by Fishery Research Services Marine Laboratory on behalf of SERAD and FSA in Scotland and for 1b on behalf of MAFF/DOH/FSA in England and Wales

A STATUTORY REQUIREMENT UNDER :

B.

- a) The Shellfish Growing Waters Directive — 79/923/EEC
- b) The Surface Waters (Shellfish) (Classifications) (Scotland) Regulations 1997
S.I 1997 No. 2470

THIS LEGISLATION REQUIRES THAT MONITORING IS UNDERTAKEN FOR :

Faecal Coliforms in Shellfish Flesh in Designated Waters

**This work is undertaken by Fishery Research Services Marine Laboratory on behalf of
SEPA in Scotland**



Marine
Laboratory
Aberdeen

C. END PRODUCT STANDARD TESTING

Chapter V of Directive 91/492/EEC requires that all shellfish placed on the market for human consumption meets certain specific criteria.

For marine biotoxins in shellfish flesh some analyses are undertaken by FRSSL on a commercial basis for processing companies throughout the UK.

D. SHELLFISH PURIFICATION SYSTEMS

Directive 91/492/EEC also requires that shellfish purification systems meet certain standards.

FRSSL also undertakes the testing of shellfish purification systems in Scotland, which is required prior to approval for use being granted.

ALGAL TOXIN MONITORING AND SURVEILLANCE

OBJECTIVES

1. to provide an early warning of the presence of algal toxins in shellfish.
2. to protect consumers by preventing toxic shellfish reaching the commercial market
3. to protect consumers from harvesting toxic shellfish for their own consumption
4. to ensure that shellfish placed on the market meets the requirements of:
 - a) the shellfish hygiene directive - 91/492/EEC
 - b) the food safety (live bivalve molluscs and other shellfish regulations) 1992
 - c) the food safety (fishery products) regulations 1992

SAMPLING STRATEGY

40 INSHORE SITES :

RISK ASSESSMENT BASED UPON HISTORICAL
TOXIN INCIDENTS AND IMPORTANCE TO
SHELLFISH AQUACULTURE INDUSTRY

HIGH RISK AREAS :

PEAK TOXIN PERIOD : APRIL TO SEPTEMBER

PSP - WEEKLY MONITORING

DSP - MONTHLY MONITORING)

ASP - MONTHLY MONITORING)

INCREASED IF PHYTOPLANKTON LEVELS RISE

LOW TOXIN PERIOD : OCTOBER TO MARCH

ALL TOXINS - MONTHLY MONITORING

LOW RISK AREAS :

PEAK TOXIN PERIOD :

PSP - FORTNIGHTLY MONITORING

DSP - MONTHLY MONITORING

ASP - MONTHLY MONITORING

LOW TOXIN PERIOD :

ALL TOXINS - MONTHLY MONITORING

PERMITTED LEVELS OF TOXIN

PSP The total PSP content in the edible parts of molluscs (the whole animal or any part edible separately) must not exceed 80 microgrammes per 100g of mollusc flesh in accordance with the biological testing method - in association if necessary with a chemical method for the detection of saxitoxin.

If the results of such tests are challenged, the reference method shall be the biological method.

DSP The customary biological testing methods must not give a positive result for the presence of DSP in the edible parts of molluscs, that is the whole body or any part edible separately.

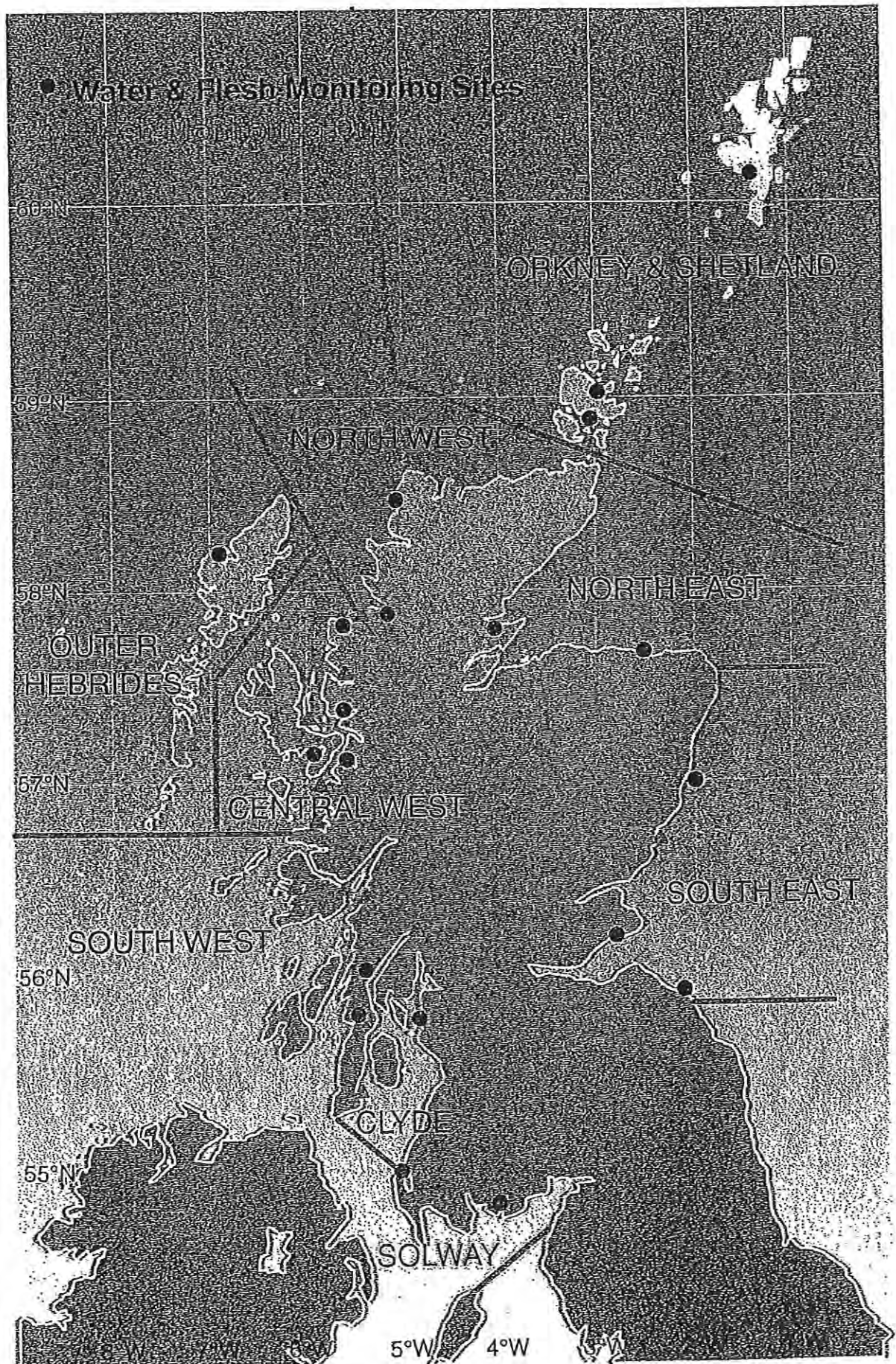
ASP The total ASP content in the edible parts of molluscs (the whole animal or any part edible separately) must not exceed 20 microgrammes per gram of mollusc flesh in accordance with an HPLC testing method.

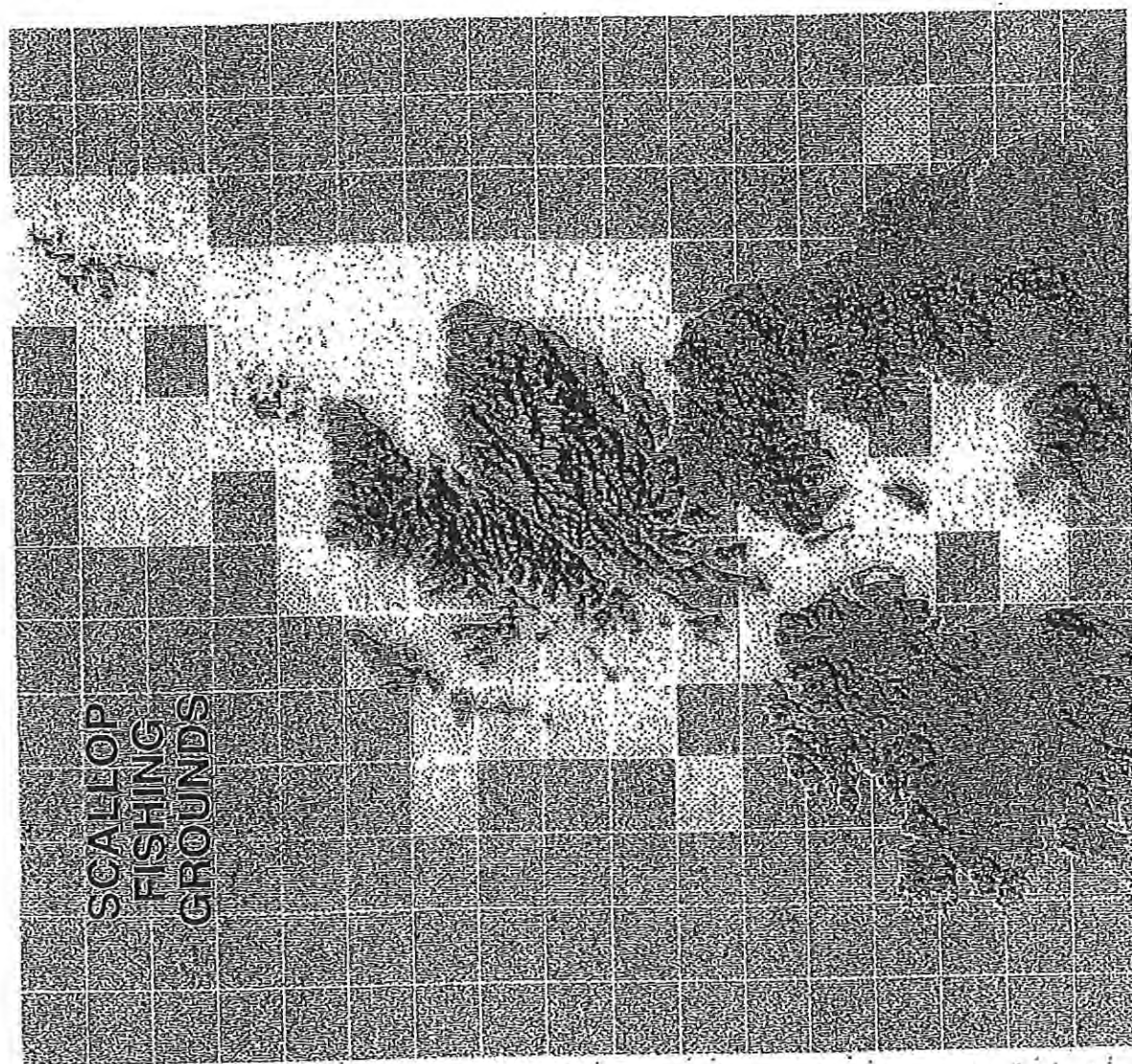
CLASSIFIED BIVALVE MOLLUSC PRODUCTION AREAS, 2000



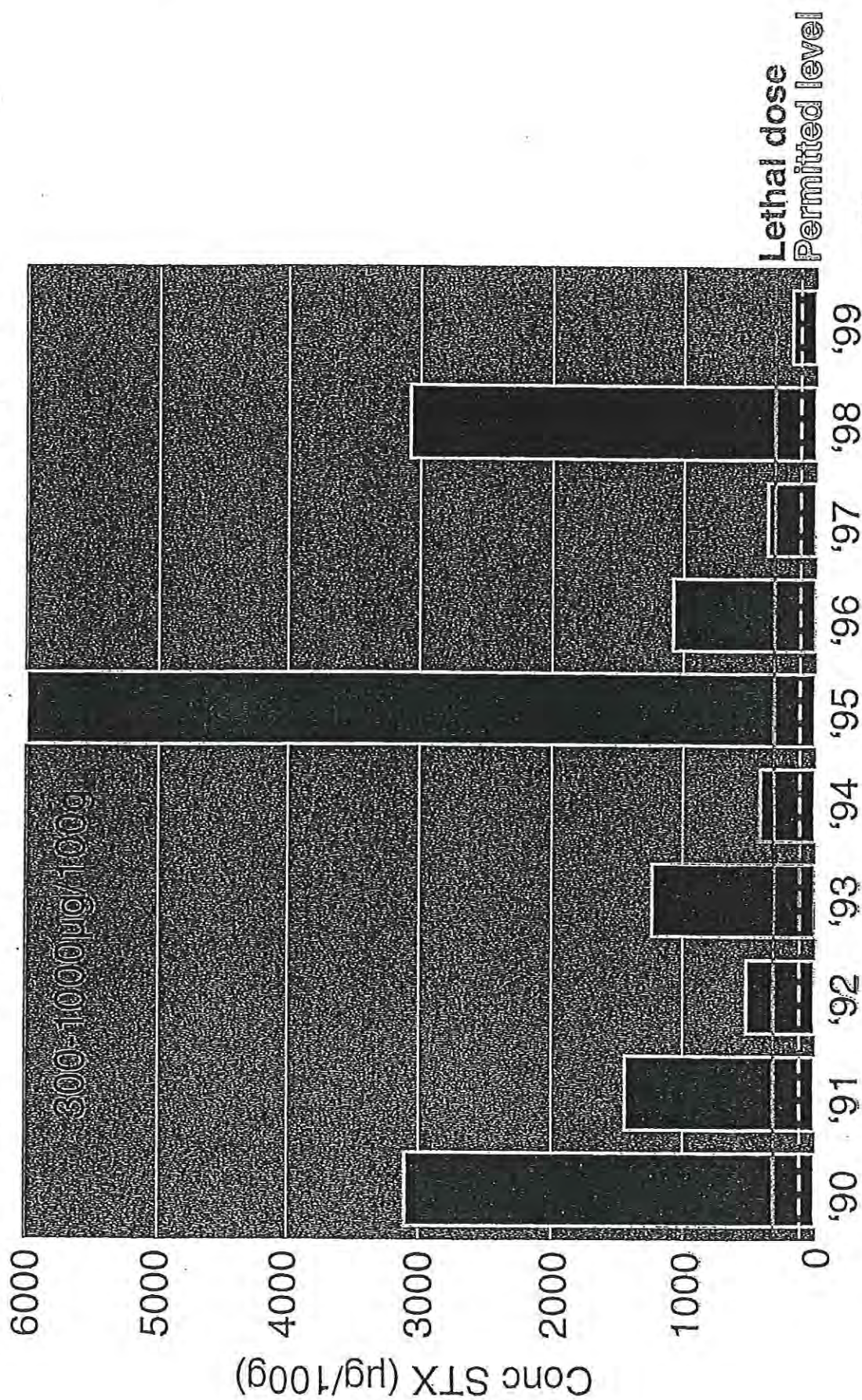
Marine
Laboratory
Aberdeen

Classification	A	A/B Seasonal	B	C	Total
LOCAL AUTHORITY AREA					
Edinburgh			1	1	1
Fife			2	2	2
Angus			1	1	1
Moray	2				2
Orkney	12	1	14		27
Shetland	4	5	2		11
Highland:					
Sutherland	3	2			5
Ross and Cromarty	6	2	3		11
Skye and Lochalsh	9	5	5		19
Lochaber	4	7	3		14
Western Isles	9	1	7	1	18
Argyll and Bute	17	13	5	2	37
Inverclyde			1	2	3
North Ayrshire	2		2		4
Dumfries and Galloway	1	1	4		6
Total	69	37	47	8	161

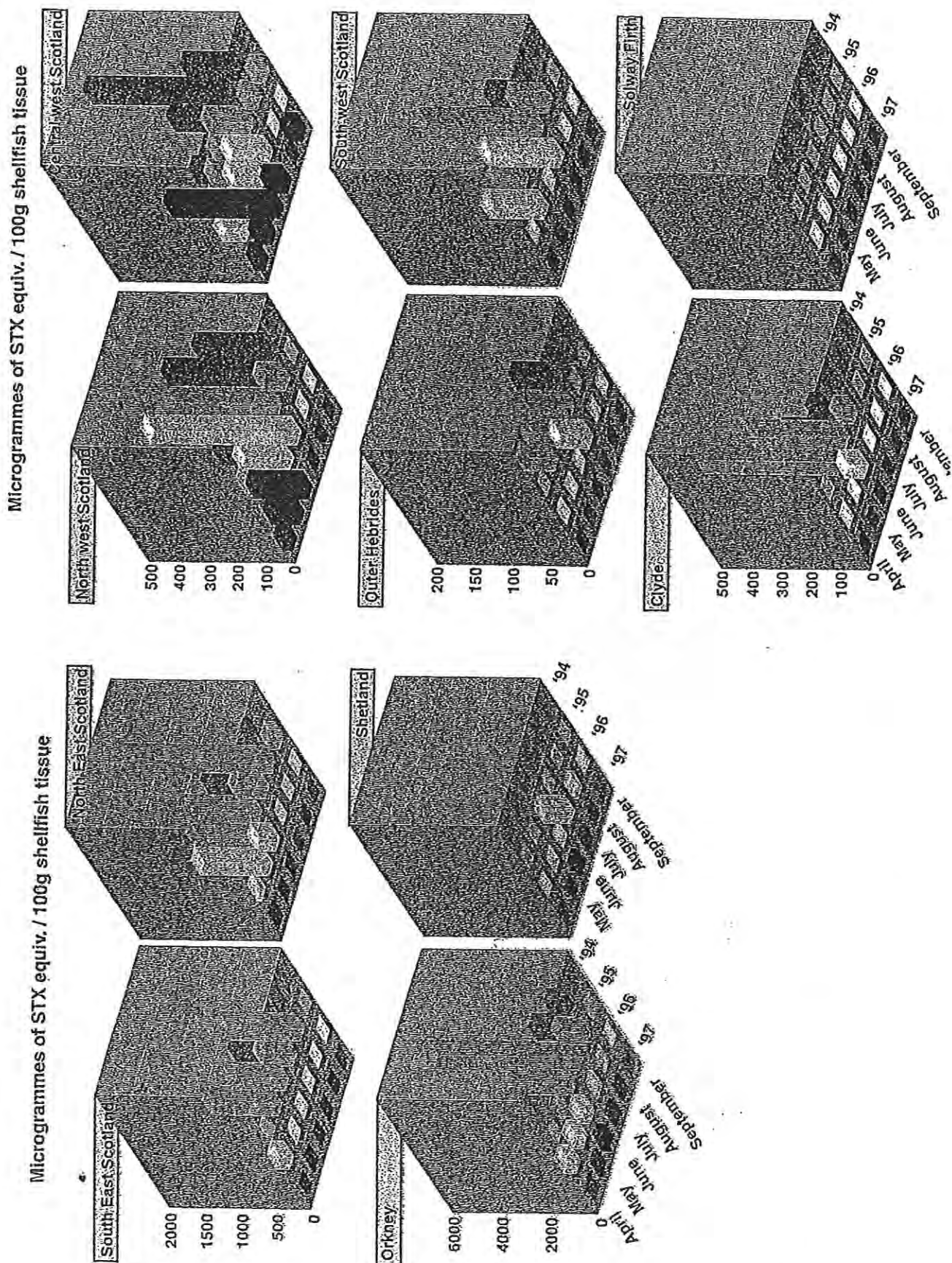




LETHAL DOSE OF SAXITOXIN

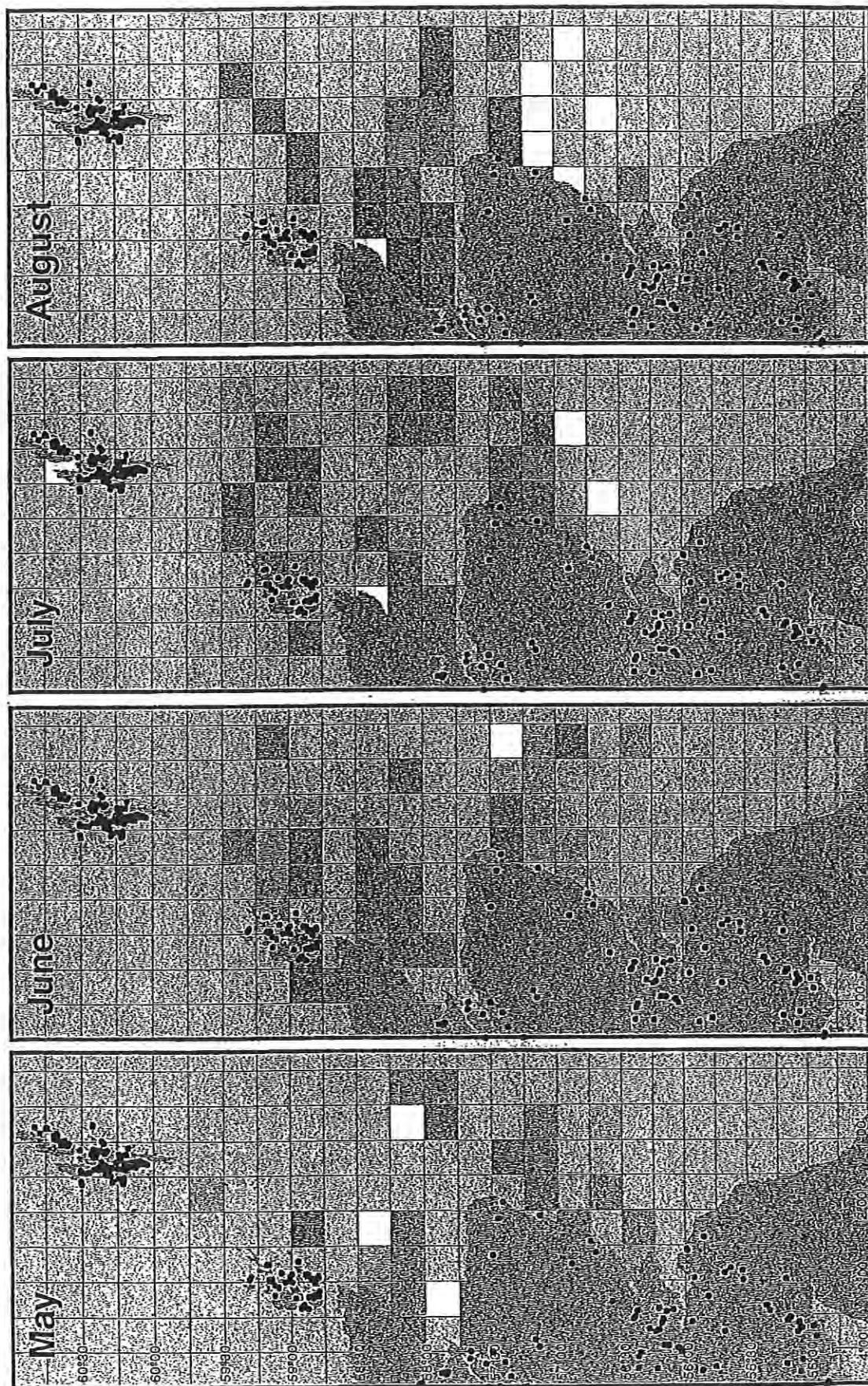


Maximum detected levels of PSP toxins in Scotland 1994-97



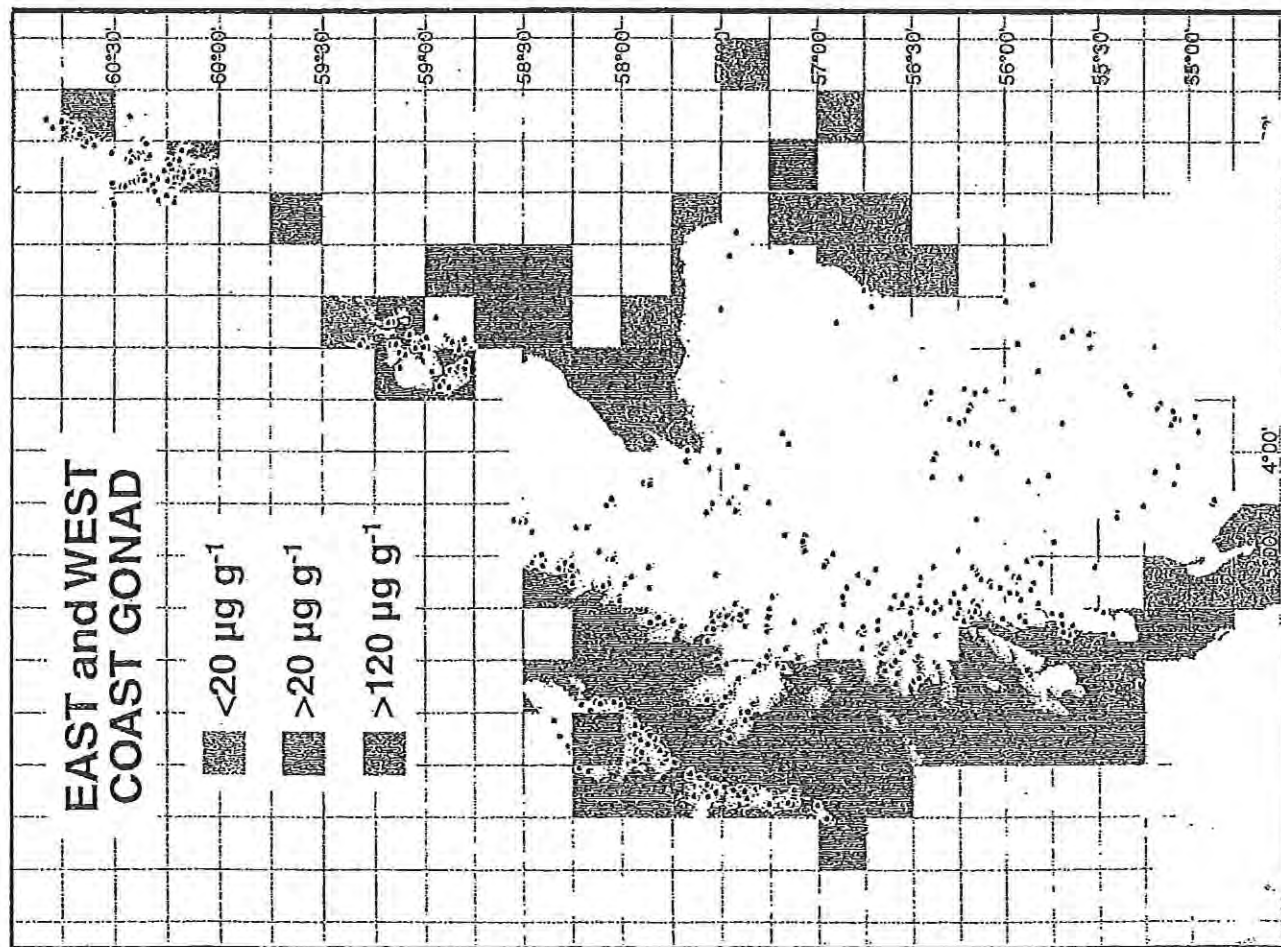
Marine Laboratory Aberdeen

PSP LEVELS IN SCALLOP GONAD EAST COAST GROUNDS 1995



Marine
Laboratory
Aberdeen

SCALLOP FISHING GROUNDS IN WHICH ASPTOXINS WERE DETECTED, 1999



Total Molluscan Landings 1996-1999 by Species (tonnes).

Species	1996	1997	1998	1999
Scallops	9539	9777	9715	8465
Queen scallop	1896	5232	6912	4962
Razorfish	58	220	115	79
Periwinkles	1620	2462	1675	1003
Cockles	86	72	110	120
Whelks	2968	1894	1074	1314
Squid	634	823	1355	1199

(1999 data provisional)

SCOTTISH BIVALVE MOLLUSC AQUACULTURE PRODUCTION

(For human consumption)

	1995	1996	1997	1998	1999 (provisional)
Pacific oysters (000s)	1,973	2,781	2,787	2,857	2,895
Native oysters (000s)	182	96	11	87	142
Scallops (000s)	300	302	223	343	127
Queens (000s)	1,147	1,271	1,207	3,676	2,842
Mussels (tonnes)	882	1,072	1,307	1,355	1,400

1999 — 237 registered active sites
137 produced shellfish

REQUIREMENTS CONCERNING LIVE SHELLFISH

LIVE SHELLFISH INTENDED FOR IMMEDIATE HUMAN CONSUMPTION MUST COMPLY WITH THE FOLLOWING REQUIREMENTS:

1. THE POSSESSION OF VISUAL CHARACTERISTICS ASSOCIATED WITH FRESHNESS AND VIABILITY, INCLUDING SHELLS FREE OF DIRT, AN ADEQUATE RESPONSE TO PERCUSSION, AND NORMAL AMOUNTS OF INTRAVALVULAR LIQUID.
2. THEY MUST CONTAIN LESS THAN 300 FAECAL COLIFORMS OR LESS THAN 230 *E. COLI* PER 100 GRAMS OF MOLLUSC FLESH AND INTRAVALVULAR LIQUID BASED ON A FIVE-TUBE, THREE-DILUTION MPN-TEST OR ANY OTHER BACTERIOLOGICAL PROCEDURE SHOWN TO BE OF EQUIVALENT ACCURACY.
3. THEY MUST NOT CONTAIN *SALMONELLA* IN 25 GRAMS OF MOLLUSC FLESH.
4. THEY MUST NOT CONTAIN TOXIC OR OBJECTIONABLE COMPOUNDS OCCURRING NATURALLY OR ADDED TO THE ENVIRONMENT SUCH AS THOSE LISTED IN THE ANNEX TO DIRECTIVE 79/923/EEC IN SUCH QUANTITIES THAT THE CALCULATED DIETARY INTAKE EXCEED THE PERMISSIBLE DAILY INTAKE (PDI) OR THAT THE TASTE OF THE MOLLUSCS MAY BE IMPAIRED.
5. THE UPPER LIMITS AS REGARDS RADIONUCLIDE CONTENTS MUST NOT BE EXCEEDED.

REQUIREMENTS CONCERNING LIVE SHELLFISH

6. THE TOTAL PARALYTIC SHELLFISH POISON (PSP) CONTENT IN THE EDIBLE PARTS OF MOLLUSCS THAT IS THE WHOLE BODY OR ANY PART EDIBLE SEPARATELY MUST NOT EXCEED 80 MICROGRAMMES PER 100 GRAMS OF THE MOLLUSC FLESH IN ACCORDANCE WITH THE BIOLOGICAL TESTING METHOD - IN ASSOCIATION IF NECESSARY WITH A CHEMICAL METHOD FOR THE DETECTION OF SAXITOXIN. IF THE RESULTS OF SUCH TESTS ARE CHALLENGED, THE REFERENCE METHOD SHALL BE THE BIOLOGICAL METHOD.
7. THE CUSTOMARY BIOLOGICAL TESTING METHODS MUST NOT GIVE A POSITIVE RESULT TO THE PRESENCE OF DIARRHETIC SHELLFISH POISON (DSP) IN THE EDIBLE PARTS OF MOLLUSCS THAT IS THE WHOLE BODY OR ANY PART EDIBLE SEPARATELY.
- 7a. THE TOTAL AMNESIC SHELLFISH POISON (ASP) CONTENT IN THE EDIBLE PARTS OF THE MOLLUSC (THE ENTIRE BODY OR ANY PART EDIBLE SEPARATELY) MUST NOT EXCEED 20 MICROGRAMMES PER GRAM USING THE HPLC METHOD.
8. IN THE ABSENCE OF ROUTINE VIRUS TESTING PROCEDURES AND THE ESTABLISHMENT OF VIROLOGICAL STANDARDS, HEALTH CHECKS MUST BE BASED ON FAECAL BACTERIA COUNTS.

CLOSURE MECHANISMS

1. Food and Environment Protection Act 1985

FEPA 1985

2. Voluntary Closure Agreement

VCA

3. Temporary Prohibition Order

TPO

TOXICITY IN SCALLOP TISSUES

Toxins concentrations vary in different tissues

In decreasing order of concentration, the ranking in scallop tissues is :

**Hepatopancreas
Mantle and gills
Gonad
Adductor muscle**

POSSIBLE FUTURE TESTING AND CONTROL REGIMES FOR SCALLOPS

1. Monitoring is undertaken using whole animal testing
2. If toxin levels in the whole animal exceed permitted level, testing is then undertaken on gonad tissue.
3. If toxicity in gonad tissue remains below the permitted level, continued fishing may be permitted, but all scallops landed must be processed, and only gonad and adductor muscle placed on the market.
4. If toxin levels in gonad tissue exceed permitted levels, then all fishing is prohibited in affected areas.

The Occurrence of Amnesic Shellfish Poisons in Scottish Waters

S. Gallacher., P. Gillibrand.,
M. Heath., P. Hess., G. Howard.,
M.C. Kelly., E.M. Macdonald &
W.R Turrell

1987, Canada

- Mussels from Prince Edward Island
- 107 people ill, 12 intensive care, 3 dead
- nausea, vomiting, abdominal cramps,
headache, diarrhoea, memory loss.
- 15 minutes to 38 hours

ASP Cause

- Distinctive mouse bioassay
- Domoic acid, water soluble tricarboxylic amino acid (311 mol wgt)
- Folk medicine: pinworm infestations
- Similar to glutamic acid, neurotransmitter in the brain
- neuronal swelling followed by death

Species detected	Listed as toxic
<i>P. australis</i>	Yes
<i>P. pungens</i>	Yes
<i>P. seriata</i>	Yes
<i>P. multiseriata</i>	Yes
<i>P. delicatissima</i>	Yes

Countries ASP detected

- Canada (1987: mussels)
- USA (1988 onwards: crabs, razor clams, anchovies)
- Portugal (1995 onwards: mussels, clams, razor clams)
- Spain (1994 onwards: mussels and scallops)
- UK (1998 confirmation: mussels, scallops, cockles, crabs, oysters)
- Ireland (1999 onwards)

Monitoring in Scotland

- Full scale monitoring commenced 1992
- Requirement of UK and EU regulations
- In most years closure of fisheries, including scallop grounds, due to PSP
- Each year PSP concentrations exceed levels thought to cause illness
- DSP detected and has caused food poisoning

History of ASP in Scotland

- 1996 and 1997 ASP suspected in shellfish from Scotland
- 1998 research project established to determine if ASP was present
- ASP detected in a range of shellfish species
- *Pecten maximus* was the most frequently affected species, with the highest toxicity
- 1999 EU regulations in place

1999 ASP programme

- HPLC method undergoing accreditation
- Analysis for 30 samples/week planned
- Positive sample obtained from off-shore scallops in July
- More intense monitoring put in place
- 150 to 300 samples/week from August
- 2166 samples for monitoring data, more for research purposes

DA analysis

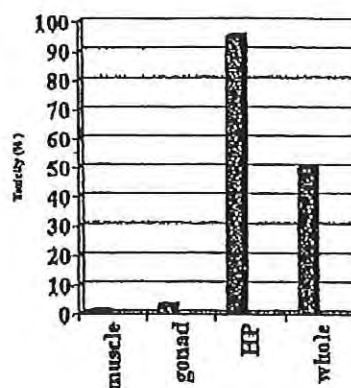
- DA analysis by HPLC-UV Quilliam *et al.*, 1995
- DA isomers present
- DA confirmed by LC-MS: 3 laboratories independently
- Inter-laboratory study: Good correlation FRS, MI and DANI
- EU validation through 2000

Regulatory limit

- PSP = 80 ug/100g = 0.80 ug/~~100~~g
- ASP = 20 ug/g = 25 x more
- Based on toxicological information from the Canadian outbreak and animal studies

Which tissue to test ?

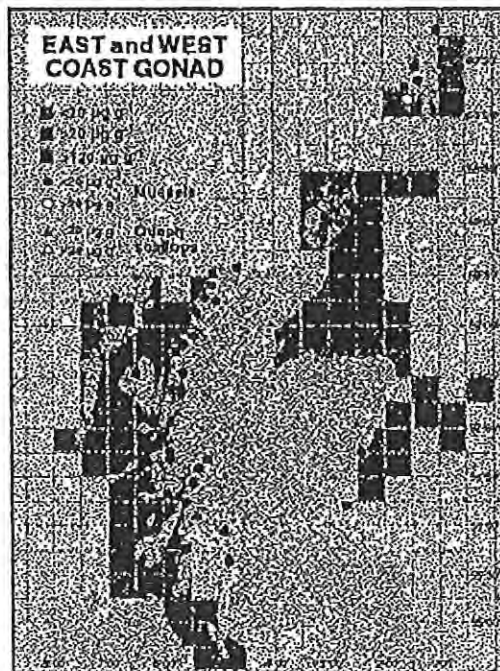
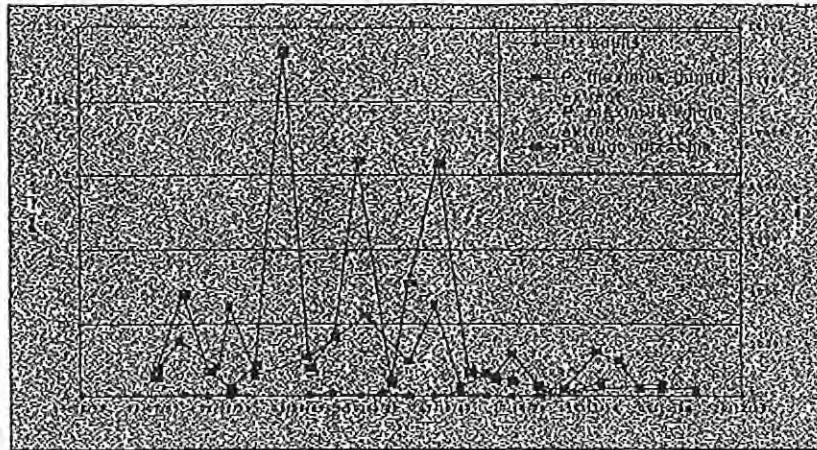
- Whole animal v's gonad
- Scotland uses scallop gonad
- Referred to EU
- Awaiting decision



Highest DA concentrations recorded (ug/g)

King scallop whole tissue	534
King scallop gonad	294
Mussels	23
Queens scallop	48
Oysters	traces

DA concentration and *Pseudo-nitzschia* nos.

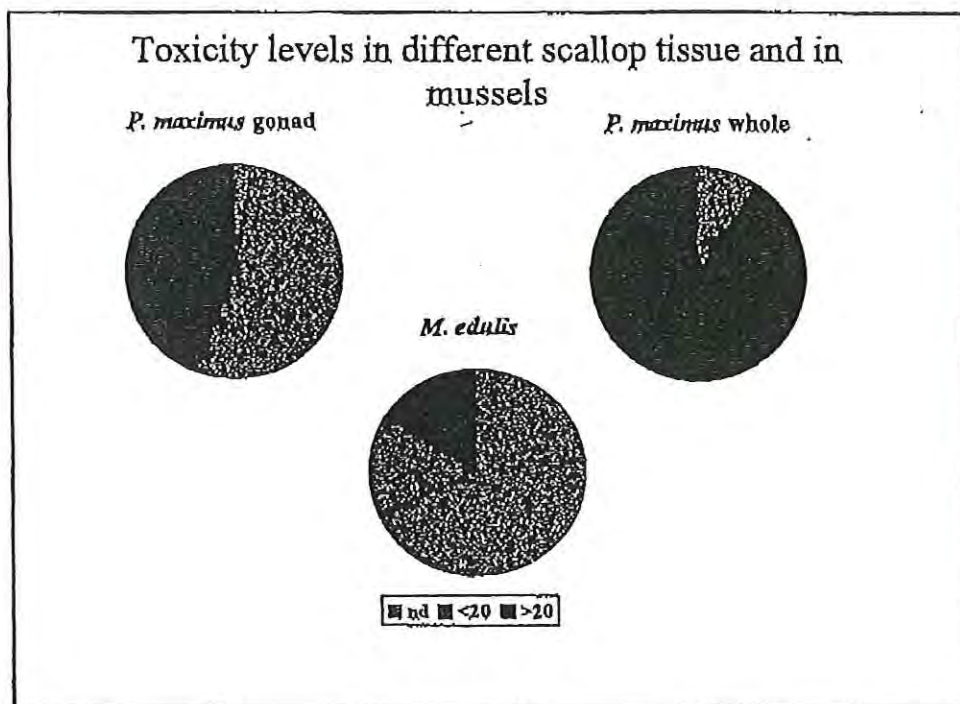


UK/Irish/EU Biotoxin Reference Labs

- EU: Vigo, Spain:
- UK: FRS, Aberdeen
- Ireland: MI, Dublin
- Standardise and validate methodology
 - UK: Inter-comparison of ASP method
 - EU: Inter-comparison of ASP method

Research issues

- Cause of off-shore scallop toxicity?
- Mechanisms of long-term scallop toxicity?
- Reasons for short-retention in mussels?
- Quicker screening techniques?



Phytoplankton : Research Priorities

Dr. Robin Raine,
Martin Ryan Institute,
National University of Ireland,
Galway.

Introduction

Over the past decade, considerable progress has been made in finding out how and why harmful phytoplankton events arise. This has to be the first step in rationalising any schemes for forecasting and prediction of these events and for minimising industry losses. Having said this, it is typical of nature that once one problem has been identified and attempts to research the problem are underway, a new, equally or more serious problem arises and in this way the research priority goalposts are continually being shifted. Take for example the "red tides" of *Gyrodinium aureolum* which first manifested themselves in an aquaculturally damaging way in 1978 and 1979. As soon as work started in researching this problem in the research-fund starved 1980's, than the problem of DSP and *Dinophysis* arose. Table 1 summaries the harmful event categories which are problems within Ireland. This paper summarises the successes and remaining unresolved problems that have resulted from the research field to date, and suggests ways in which resources could be applied in tackling outstanding problems.

Table 1. Harmful phytoplankton events around Irish coasts.

Species	Nature of problem	Effect	Main areas affected
<i>Gyrodinium aureolum</i>	Red tides	Finfish and shellfish mortalities, probably arising from suffocation and/or toxin	Southeast, south and southwest coasts. Donegal Bay
<i>Dinophysis acuta</i> <i>Dinophysis acuminata</i>	DSP toxins (Okadaic acid; DTX-2)	Shellfish contamination with toxin	Southwest
<i>Alexandrium tamarense</i>	PSP toxin	Shellfish contamination with toxin	Cork Harbour
<i>Pseudo-nitzschia</i> spp.	ASP toxin (Domoic acid)	Shellfish contamination with toxin	Entire west coast
? (unknown) ?	AZP	Shellfish contamination with toxin	Entire west coast

Phytoplankton and Water Circulation

The microscopic, single celled phytoplankton have virtually no independent means of movement in the sea and are consequently carried around in the sea with local currents. It is therefore useful to summarise the general current regime around the Irish coast, particularly for the aquaculturally important south and west coasts. This is shown in Figure 1, where two important features should be pointed out. The first is the position of the Irish Shelf Front which runs along the entire western coast. Just as fronts depicted in weather forecasts are boundaries between differing types of air (e.g. warm and cold), this front is literally a boundary between two types of seawater : Irish coastal water inshore of the front and very salty oceanic water further offshore.

The second thing to note in Figure 1 is the proximity of the Irish Shelf Front to the coastline off the southwest of Ireland and off Erris Head (Co. Mayo). We now know that when winds are blowing from the west or southwest, oceanic water is blown towards the coast and the Irish Shelf Front is located even further in on the shoreline. This has the net effect of cutting off the generally northwards, or clockwise, flow of the coastal water inside the front at these two points. As winds are generally from the southwest quarter, the overall effect is the frequent formation of two gyres or large eddies off the south and west coasts (Figure 2). When winds from the west or southwest stop, then the circulation pattern switches from the one depicted in Figure 2 to that in Figure 1. This has considerable consequences on phytoplankton as local conditions in the gyres are ideal for the development of dinoflagellates such as *Gyrodinium* and others.

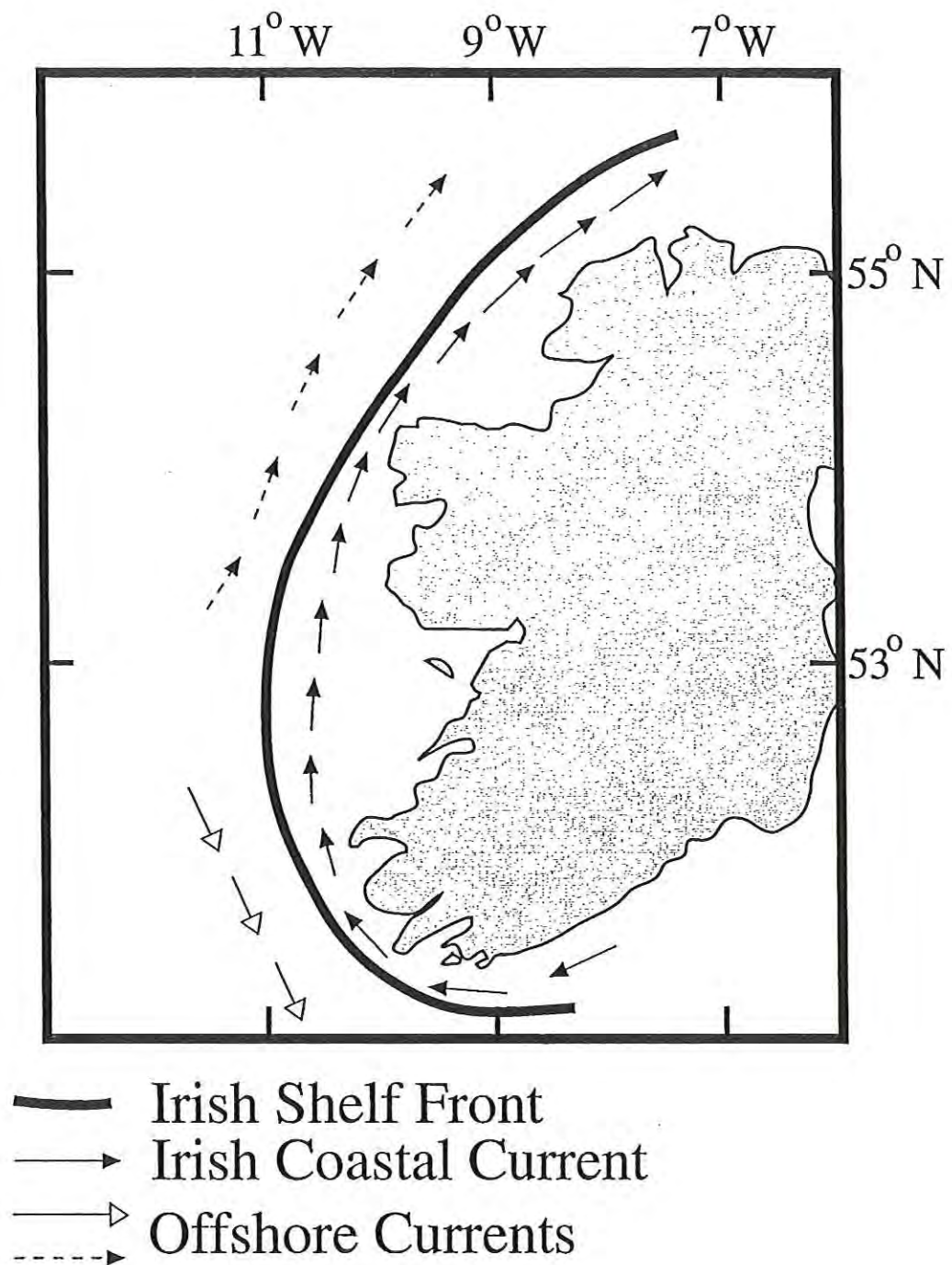


Figure 1. General surface current pattern off the west of Ireland based on moored current meter data and satellite imagery. (Compiled from Pingree & Le Cann, 1989; Huang et al., 1991; Raine et al., 1994; Raine & McMahon, 1998; Raine et al., 2000)

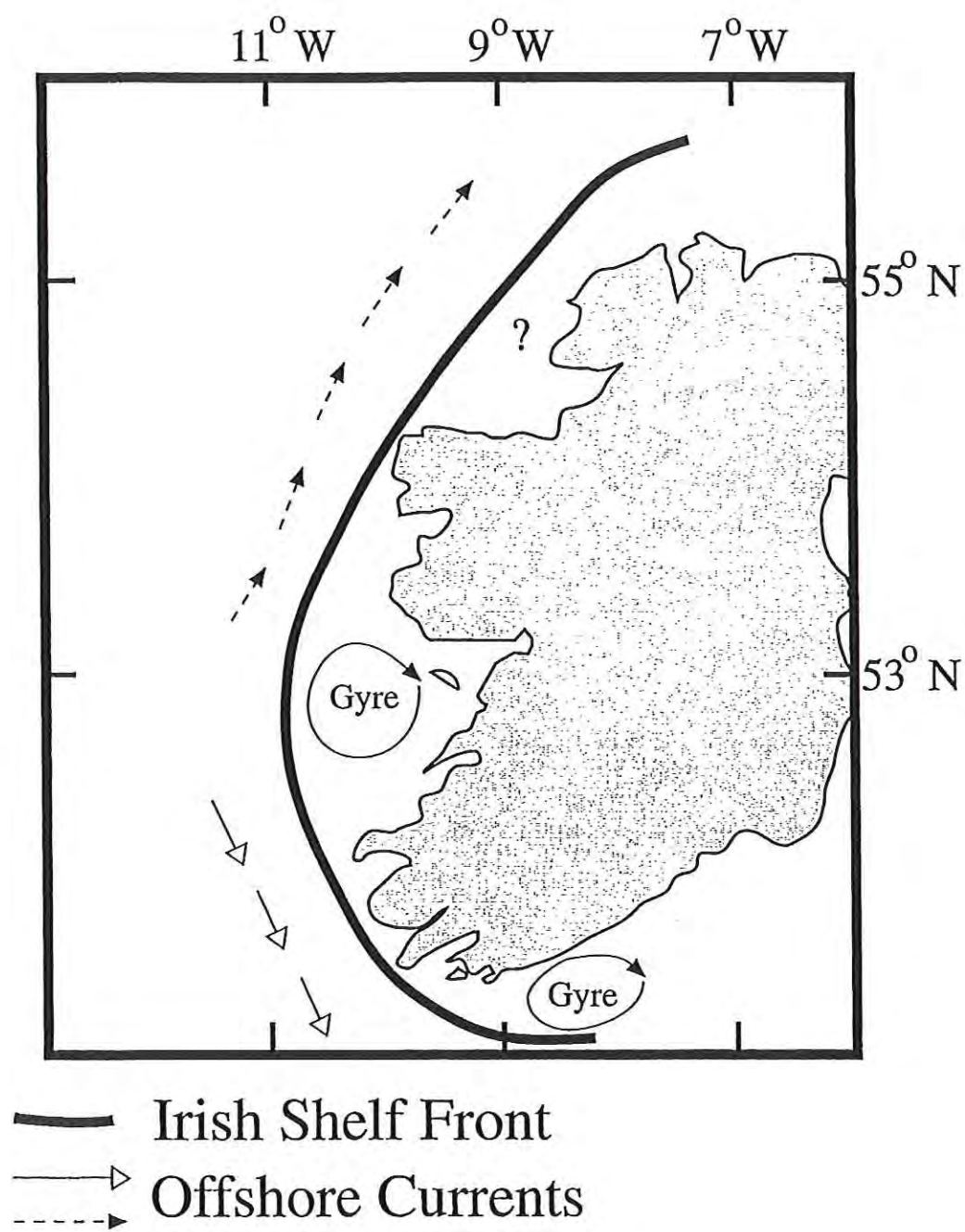


Figure 2. Water circulation patterns under west or southwest wind conditions.

Gyrodinium aureolum and red tides

Red tides of *Gyrodinium* have arisen periodically around the Irish coast since the mid-1970s. Earlier ones may have occurred but these have not been recorded. During summer, massive sub-surface populations develop at about 25-30 m depth in the gyre region off the south coast. and, presumably, off the west coast. Extensive sampling of the western gyre has only just begun.

When winds from the west or southwest stop or are reversed, circulation patterns switch from that in Figure 2 to that in Figure 1, and the dinoflagellates which have developed in the gyres are carried around to the southwest to the mouths of the large bays of SW Ireland. A resumption of southwest winds blow water with these plankton into the bays.

The movement of populations from off the south coast right up to Whiddy Island in Bantry Bay takes only 3-4 days. It is totally dependant on wind direction, does not require a particularly unusual sequence of shifts in wind direction, and explains why red tides appear so rapidly in the bays of southwestern Ireland. In terms of harmful phytoplankton events, the scenario described is only really applicable to the summer (July-September) when other conditions in the sea such as water temperature are suitable for the development of dinoflagellates. The basic mechanism is also responsible for the red tide in Sligo Bay in 1992 which was accompanied by extensive mortalities of farmed clams.

Water and phytoplankton exchange in the bays of the southwest.

Prevailing winds from the southwest quarter are roughly aligned to the axis or shape of the large bays of southwestern Ireland, Bantry Bay, Dunmanus Bay and Long Island Bay. Variations in the wind strength result in quite extensive water exchange with these bays and the water just outside them. The mechanism involved is depicted in Figure 3. the exchanges are quite considerable in a local context. For example, we have measured water exchanges of ca. 70% of the volume in Bantry Bay with the open sea in the space of 2-3 days as a direct consequence of wind-forced exchange.

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***Dinophysis* and DSP**

DSP problems have had a fairly frequent occurrence in the southwest in the summer of past years. Linked to the presence of *Dinophysis acuta* and *D. acuminata*, an unusual aspect of these harmful events has been the occasional persistence of the presence of toxins in shellfish tissue. Although why toxicity persists is not yet understood, the onset of shellfish tissue contamination is linked directly to exchanges of phytoplankton within the bays of the southwest with those outside, in exactly the same mechanisms as for *Gyrodinium aureolum* outlined above.

***Alexandrium* and PSP**

Phytoplankton which produce PSP toxin are geographically limited around Ireland to inside Cork Harbour. Here, there exists a resident population of *Alexandrium tamarense* which has not spread to other locations. The lifestyle of this organism includes an encystment stage whereby the cells overwinter in the sediments. The appearance of the organism in summer seems to be one of the most regular and predictable features of potentially harmful phytoplankton events around Ireland.

How the population arose initially is open to question. At present, there is considerable interest in the transmission of harmful species via dormant cysts in the ballast water of commercial shipping. Whether or not this is how the Cork Harbour population arose, it would seem that there exists a threat whereby shipping from this region taking on ballast water could spread the organism to other locations within or outside of Ireland, depending on destination.

***Pseudo-nitzschia* and ASP.**

Amnesic Shellfish Poisoning (ASP) has been a relatively recent phenomenon. It is caused by the toxin domoic acid which is produced by diatoms of the genus *Pseudo-nitzschia*. There has been considerable debate about which species of *Pseudo-nitzschia* produce domoic acid and which do not (there are more than 20 of them). For example, whereas a particular species might produce domoic acid in the northwest Atlantic, a population of the same species in the northeastern Atlantic may not have produced any toxin. The problem is exacerbated by the difficulty in identifying individual species of *Pseudo-nitzschia* which involves acid cleaning of the cells

as the local weather forecasts. Although nowadays these are quite good for 3-4 days ahead, they do not appear to have the required reliability for a more extended period.

The Irish Marine Institute have commenced a collaborative study with NOAA (National Oceanic and Atmospheric Administration) in the US. This study has the aim of improving our ability to forecast and predict harmful phytoplankton events. Although still in a pilot phase, the project is initially aiming at improving our ability to model coastal circulation patterns. These models can then be applied to forecast movements of phytoplankton in the sea.

2. *Alexandrium* and PSP

As stated earlier the PSP problem around Ireland is very localised at present, restricted to Cork Harbour. It would seem logical that every effort should be made to ensure that the problem is not spread further afield. The most likely method whereby the organism can be further distributed as a result of man's activities is through transport in ship ballast water. Thus every effort should be made in an attempt to control and restrict the organism, a process which will also require careful monitoring.

3. *Pseudo-nitzschia* and ASP

We still do not understand fully which species of the genus *Pseudo-nitzschia* produce domoic acid, and neither do we know if environmental conditions are important for the production of the toxin. Research into examining these problems must be continued. Although not based on hard evidence, there is a suspicion within the scientific community that domoic acid production is linked to the nutrient status of the *Pseudo-nitzschia* cells. It is unlikely, however, that only one factor, environmental or otherwise, contributes to domoic acid production.

Problems related to *Pseudo-nitzschia* demand an upward shift in technological development. Due to the difficult, expensive and time-consuming identification procedure for this group of organisms, monitoring can be greatly facilitated through the development of species-specific fluorescent probes. Use of these probes, which is a developing technology, have had some success in the US, notably through the work of Chris Scholen and his group in California. It would be extremely beneficial to transfer the technology over to Ireland.

“Biotoxin Sampling Regime in Ireland, 2000 and Beyond”

Presentation at Marine Science Biotoxin Workshop,

Dr. Catherine Butler, BIM

As a short introduction or backdrop to where we are today. In Jan 1999, the Industry was subject to EU wide health alert. Mussels that were harvested and processed between June-Sept 1998 were found biotoxin positive in France. The question has to be asked why and how did this happen? It is important not to let this happen again. As a result of this incident, The Biotoxin Liaison Group was formed. Also formed was the **Molluscan Safety committee**. This committee reviewed the existing system and made draft recommendations for improvement. As a direct result of Molluscan Safety committee recommendations, 2 shellfish coordinators were appointed by BIM.

As a shellfish coordinator, our primary function is to improve the biotoxin-monitoring programme. We carried out a risk assessment of the current biotoxin programme and identified 5 questions or areas that need to be addressed.

- 1) Where to sample from?
- 2) When should a sample be taken?
- 3) Who takes the sample?
- 4) How is the sample taken, packaged, transported
- 5) Traceability and quality control of samples

This evening, I will describe what we have carried out in relation to these 5 areas or questions.

2. When should a sample be taken?

On examining biotoxin results from previous years, it is clear that there was not always consistency in sample taking. The bottom line is that shellfish must be sampled weekly for biotoxin testing if harvesting is occurring.

Basically for DSP testing, Samples need to be taken 2 weeks prior to harvesting and then weekly during harvest.

PSP: With PSP, testing must be carried out weekly in cork harbour. during May to sept and then randomly in other areas

ASP: ASP must be tested for on a weekly basis when harvesting scallops.

From a coordinating point of view this means that we need to know the harvest season for each area.

Action: For each production area a complete list of species and typical harvest season is being determined. Once this is completed then a schedule of shellfish sampling for biotoxin testing can be drafted for an area. From this schedule of sample collection the labs are expecting samples and a checking procedure could easily be implemented by checking with the labs samples received for testing and the schedule of samples expected. If a sample is not received from an area it could be flagged and then followed up by checking with the sampler from that area. All that would be needed is a phone call to the sampler to say why is their no sample sent in this week? Is there no harvesting for some reason? If there was harvesting then a phone call to say look no sample has arrived in to the lab this week so far can you make sure on is collected and sent in ASAP.

3. Who takes the sample?

Currently a variety of people from the DoMNR, health board, semi states and industry are involved in sample collection. In the some production areas, a designated sampler responsible for shellfish sampling is known. However this is not true for many other areas. What happens if the person who usually takes the samples in a bay goes on holidays or is sick?. A sample still needs to be taken. Who is the backup sampler in cases such as this?

The person who collects samples for an area need to be known to everyone in that area. For example in Bantry Bay we know that the Bantry Bay Mussels Ltd. takes care of sampling for that area. And within Bantry Bay production area there are 13 sub areas. What happens if they are not buying from a sub area. Why should they then sample from a sub area? There is already agreement that Bantry Bay will send samples to Cork for any producer in that area once they drop a sample to their factory first thing on Monday morning. This sort of local agreement needs to be in place for all production areas.

Action: Currently a registrar of samplers is being drawn up for all production areas. All details including samplers contact number, address and a designated backup sampler who will take samples should the main sampler be sick or gone on holidays.

4. How is the sample taken, and prepared?

2) Define the Harvest season for an area, so that a sampling schedule for that area can be drafted.

3) Updating of the registrar of samplers and then training of samplers through a series of local workshops.

4) Implementing and sample traceability by introduction of the proposed coding system.

Thank you.

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Attendance	
Butler: Dr. Catherine, BIM	
Burnell: Dr. Gavin, Zoology Department, University College Cork.	
Condon: Jim, Senior Sea Fisheries Officer, DoM & NR	
Clarke: Dave, Biotoxin Unit, Marine Institute	
Cusack: Caroline, National University of Ireland, Galway.	
D	
E	
Furey: Dr. Ambrose, Ecotoxicology Department, Cork Inst. of Techn.	
Flanagan: Andrew, BioResearch Ireland, Galway.	
Forde: Tony, BioResearch Ireland, Galway.	
Ferns: Mary, Manager, South East Shellfish Co-op., Passage East.	
Flynn, Richie, Irish Shellfish Association.	
Gallagher: Dr. Susan, Senior Scientist, Marine Lab., Aberdeen.	
Greene: Tim, Seal Harbour Enterprises, Glengarriff, Co. Cork.	
Gallagher: Gareth, MD, Atlanfish Ltd., Carndonagh, Co. Donegal.	
Howard: Godfrey, Shellfish Hygiene Group, Marine Lab., Aberdeen	
Harrington: John, Kush Seafarms Ltd., Ardgroom, Co. Cork.	
Harty: Ray, Dungarvan Shellfish, An Rinn, Dungarvan	
Houlihan: Eugene, Ballindereen, Kilcolgan, Co. Galway.	
Hannan: Mary, South West Shellfish, Killorglin, Co. Kerry.	
Hayes: Eamonn, South East Shellfishmen's Association.	
James: Dr. Kevin, Ecotoxicology Dept., Cork Inst. of Technology.	
Keogh: Pat, Chief Executive, BIM.	
Kingston: Brendan, Rosscarbery Oysters, Rosscarbery, Co. Cork.	
Keilty: Kevin, South East Shellfishmen's Association.	
Lyons: Jim, Sea Lyons Seafood, Carrigaholt, Co. Clare.	
Lane: Sara, West Cork Shellfish Ltd., Myrtleville, Co. Cork.	
Luciano: Paulo, Food & Veterinary Office, Euro. Commission, Dublin	
Maguire: Donal, Aquaculture Development Manager, BIM.	
Minihane, Denis, Environment & Quality Section, BIM.	
Murphy: John, MD, Fastnet Mussels Ltd., Bantry, Co. Cork.	
Mulloy: Andy, MD, Connemara Seafoods Ltd., Westport, Co. Mayo	
Murphy: Michael, Aquaculture Initiative Team, Dundalk.	
McMahon: Dr. Terry, Section Manager, Marine Institute.	

List Of People Who Registered On The Day.

Birmingham: Francis, DoM & NR
Buchanan: Catherine, Coastal Resources Centre, UCC
Callanan: Kevin, C.I.T.
Carney: Eimear, C.I.T.
Flannery: Kevin, DoM & NR
Gallagher: Dominic, DoM & NR, Cork.
Gillman: Marian, C.I.T.
Hewson: Kevin, DoM & NR
Hugh-Jones: Tristen, Atlantic Shellfish.
Jules: Catherine – Journalist from France with Donal Maguire
Kane: Marian, National Diagnostic Centre, UCG (Bio Research)
Keaveney: Sinead, Bio Research
Kilcoyne: John, Killary Harbour.
Miles: Chris, New Zealand.
Minihane: Paddy, Bantry Fishermen's Co-op
O'Shea: Cliona, DoM & NR, Castletownbere
O'Shea: Conor, DoM & NR, Dunmore East.
O'Sullivan: Finian, Bantry Fish Farming Co-operative
O'Sullivan: Margaret, Public Analyst Lab., Galway
Scanlon: Pat, DoM & NR, Cork.
Slattery: Deirdre, M.I.
Twomey: Marie, Bantry Bay Mussels
Whelan: Peter, Food Safety Authority

26 April 2000.

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Birmingham: Francis, DoM & NR
Buchanan: Catherine, Coastal Resources Centre, UCC
Callanan: Kevin, C.I.T.
Carney: Eimear, C.I.T.
Flannery: Kevin, DoM & NR
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