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SMR 1550 - 16

WORKSHOP ON THE USE OF RECEPTOR BINDING ASSAY (RBA)

1 - 5 September 2003

Co-organized by the International Atomic Energy Agency (I.A.E.A.)

MARINE BIOTOXINS - OCCURRENCE IN SCOTTISH WATERS

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FISHERIES RESEARCH SERVICES

Marine Biotoxins - Occurrence in Scottish Waters

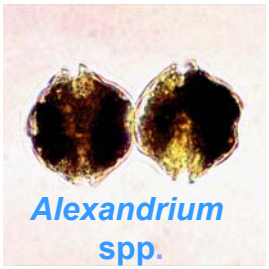
Shellfish Toxins and Industry

In Scotland

- 29 million Euros of wild shellfish are landed (scallops)
- 6 million Euros shellfish are farmed (mussels, oysters)
- 64 million Euros processed market

Three Main Shellfish Toxins

Diarrhetic Shellfish Poisoning Toxins (DSP)



Paralytic Shellfish Poisoning Toxins (PSP)

Amnesic Shellfish Poisoning Toxins (ASP)

Monitoring Since 1990

- PSP and DSP toxin monitoring extended in 1990
  - Requirements of EC Directive 91/492/EEC
- ASP toxin monitoring commenced in 1998
  - EC legislation (Council Directive 97/61/EC)
- Sampling for potential toxin producing species began in1996

Occurrence of shellfish toxins?

- Summary of available data
- Qualitative assessment
- Conclusions for monitoring programmes
- Requirement for alternative tests

Summary of available data

Toxins - Numbers of analyses

Scottish Shellfish Toxin Database

40202

PSP Bioassay
19434

Scallop - Gonad
3380

Scallop - Whole Animal
1514

Mussel - Whole Animal
9068

DSP Bioassay
6364

DSP Chemical
1513

ASP Chemical
12890

Scallop - Gonad
4588

Scallop - Whole Animal
2875

Mussel - Whole Animal
2930

Phytoplankton - Numbers of analyses

Scottish Phytoplankton Database

5095

***Alexandrium* spp.**

1062

***Pseudonitzschia* spp.**

3290

Sea Temperatures - Numbers of analyses

Scottish Monitoring
At Toxin Sites

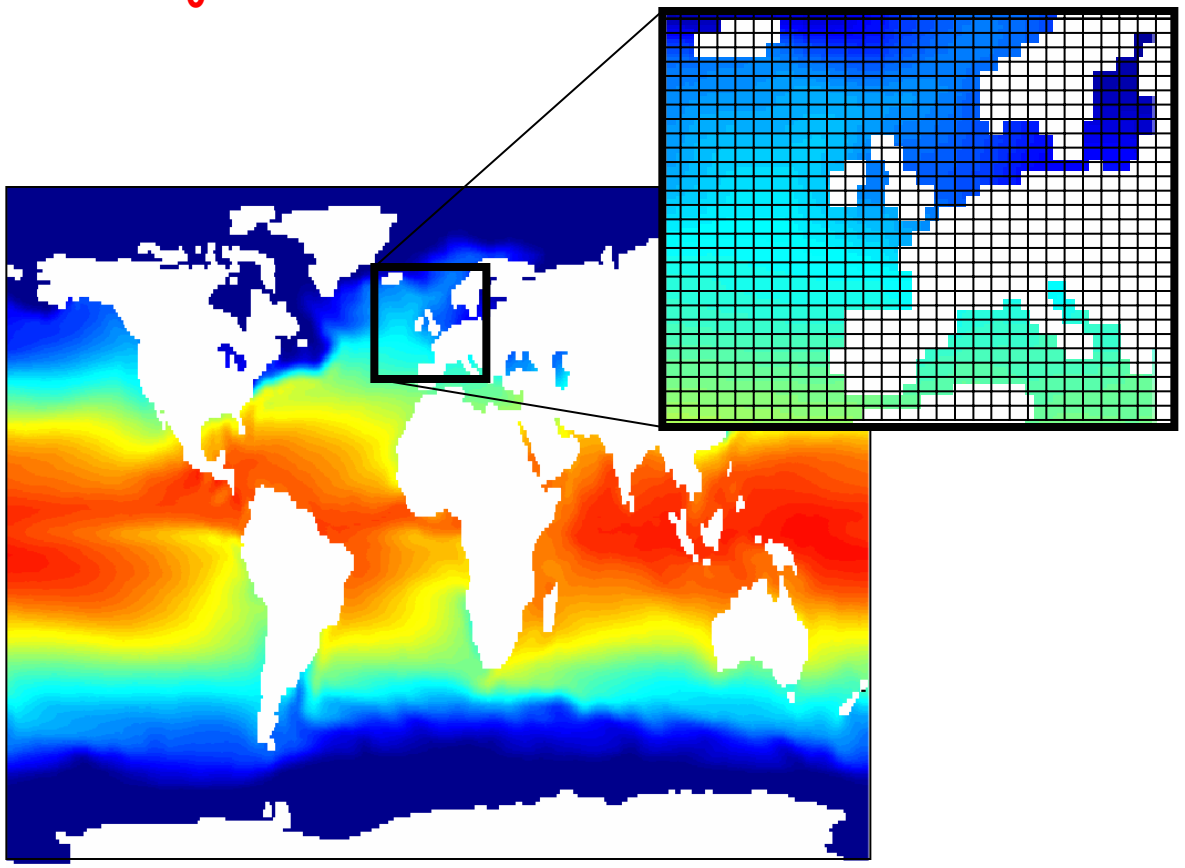
0

Reynolds Global Data Set

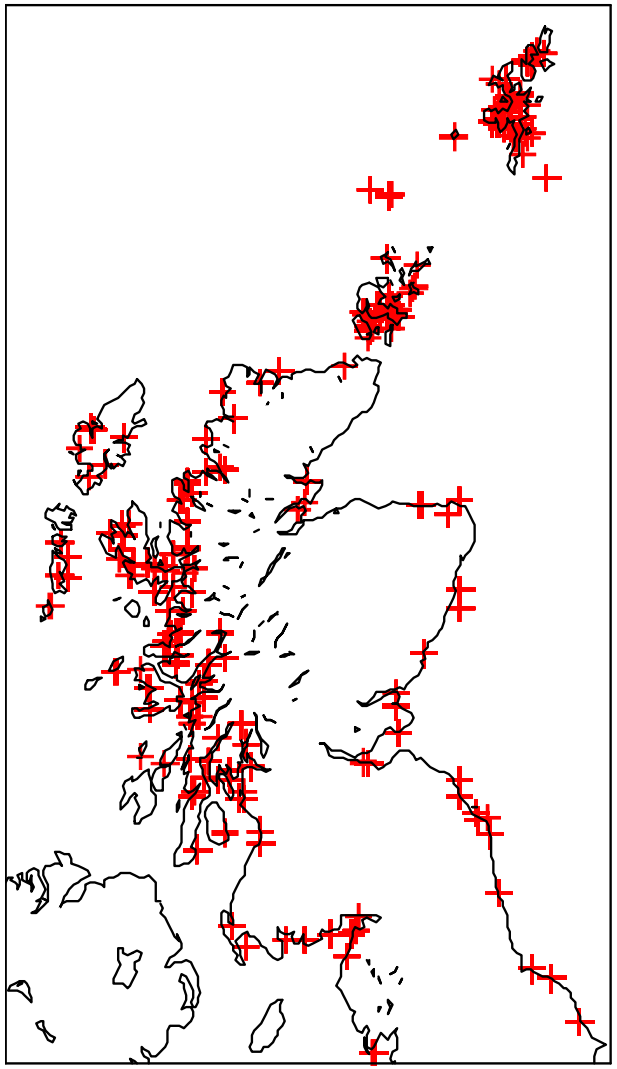
Monthly

2 degree resolution

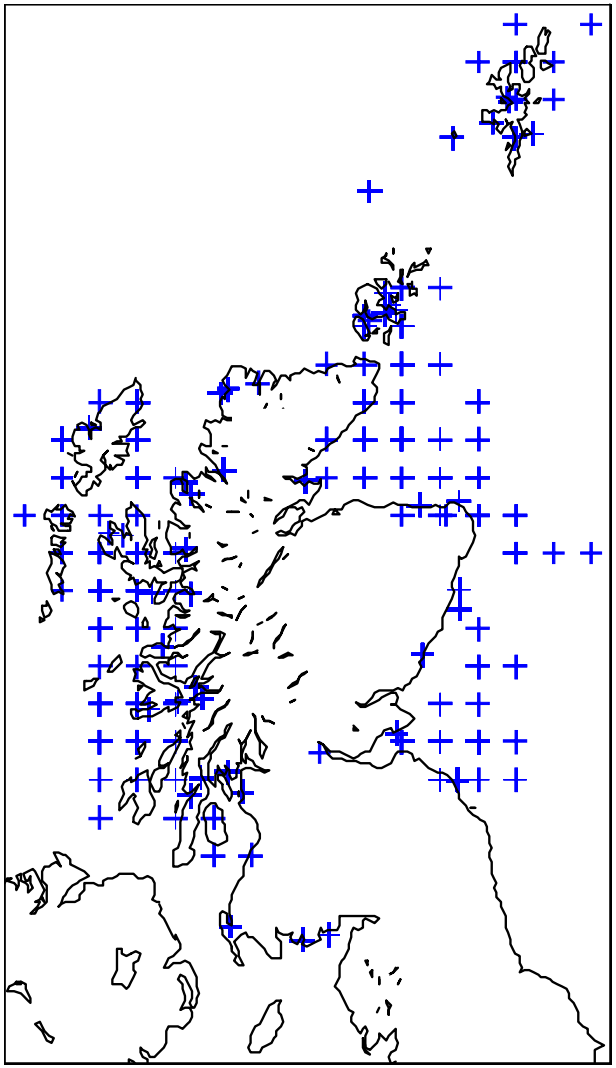
Satellite and *in-situ*



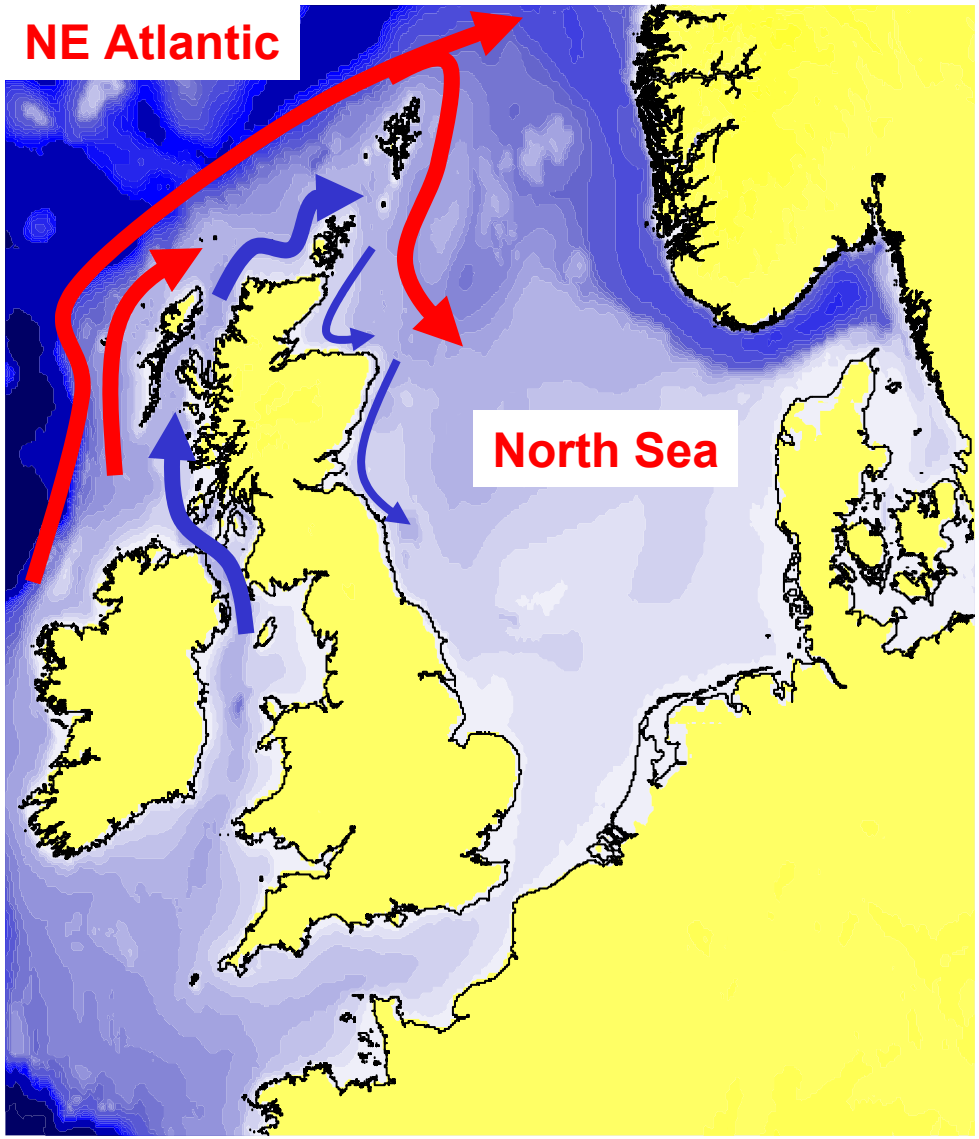
PSP Toxin (Mussel) Samples - Geographical distribution



Phytoplankton samples- Geographical distribution

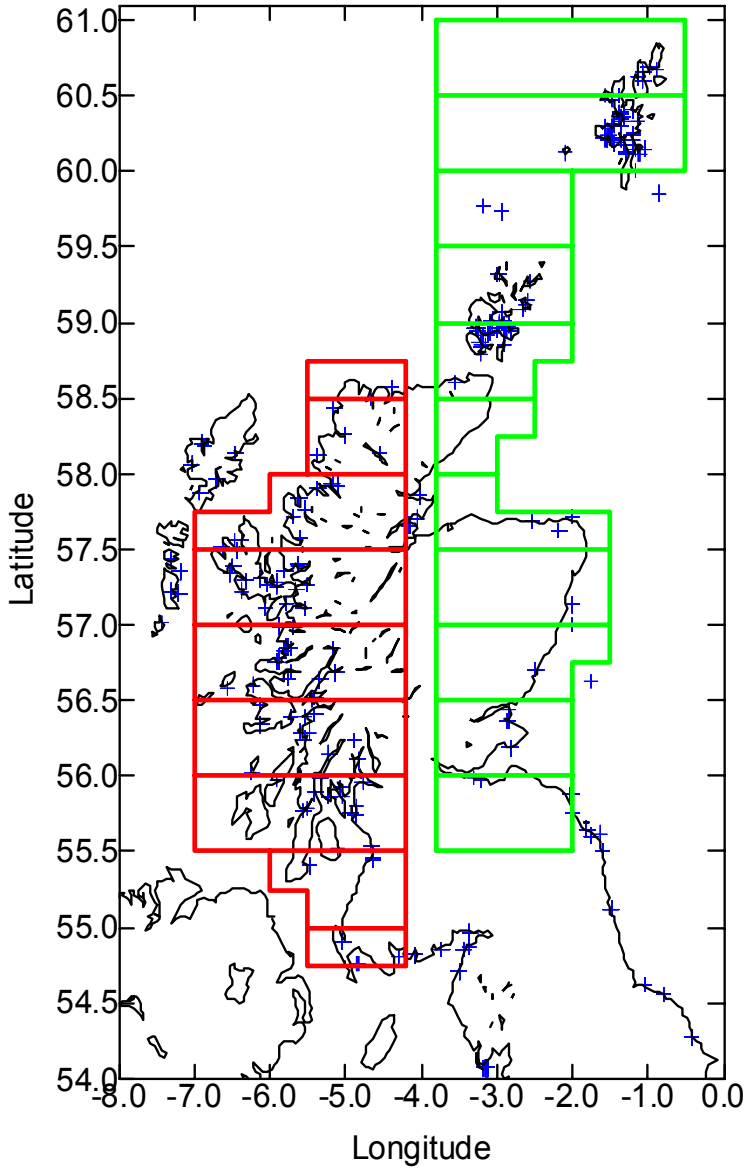


Orientation … Scottish waters

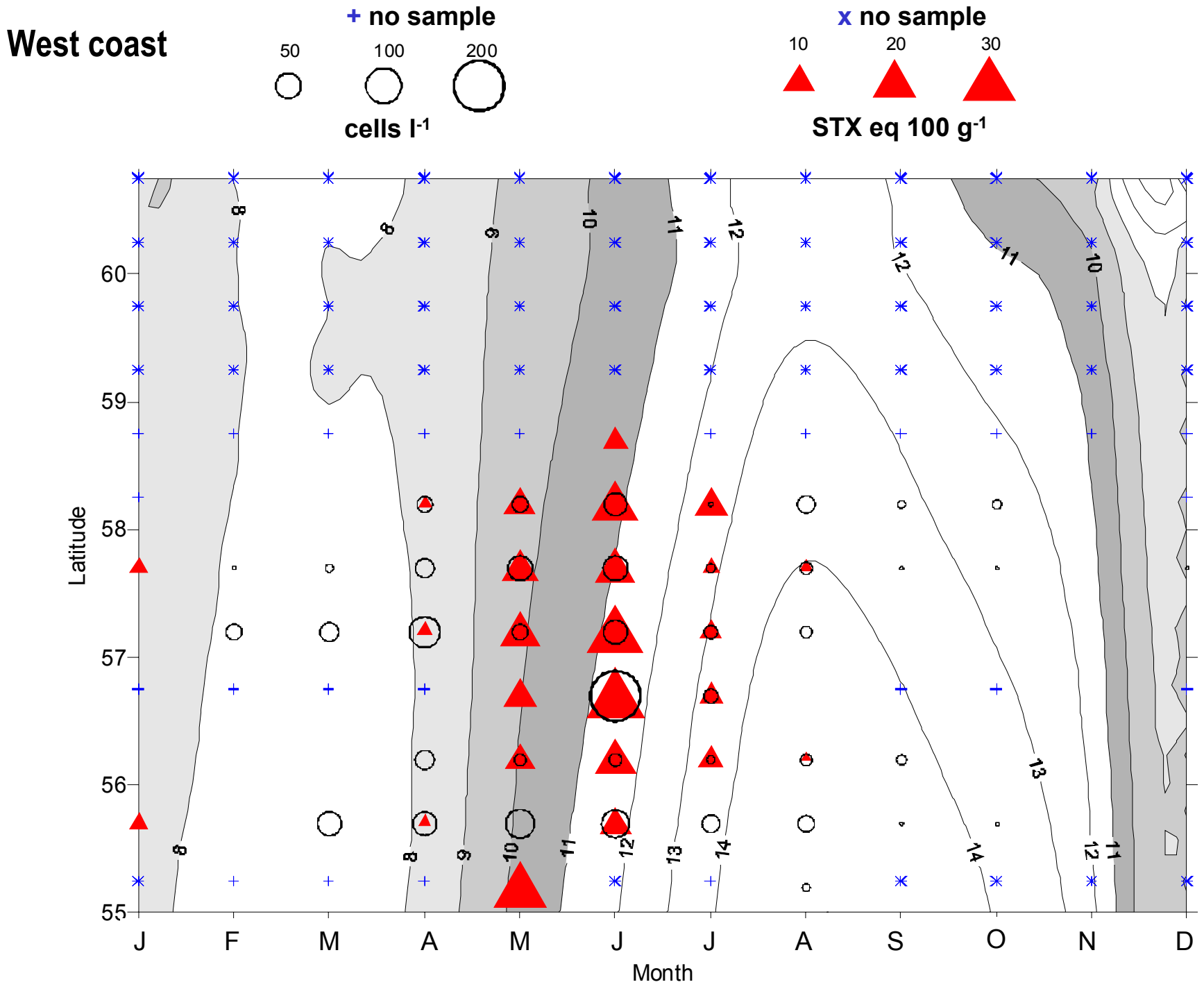


Spatial Averaging

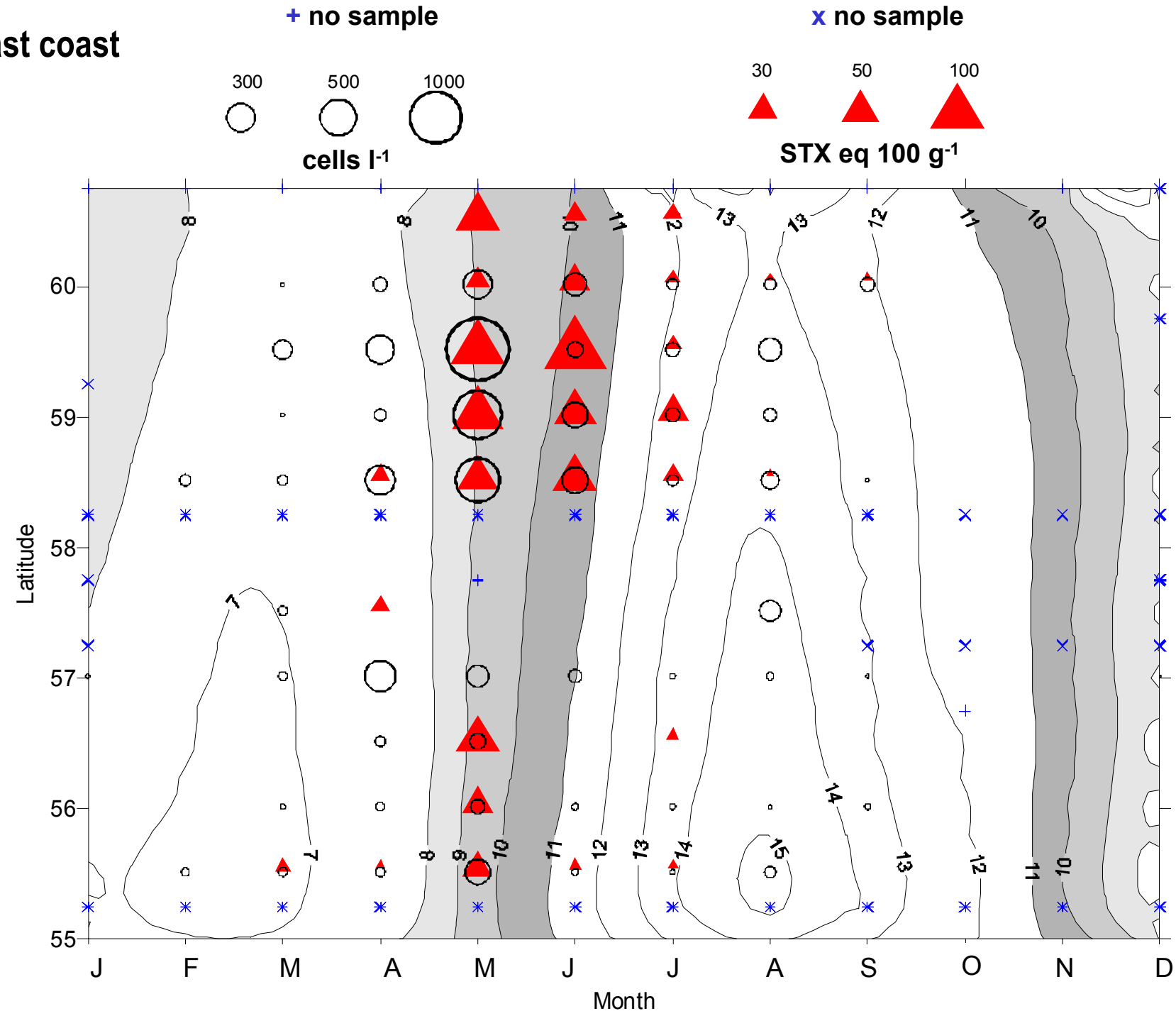
- **Same zones used for:**
  - **Shellfish**
  - **Phytoplankton**
  - **Temperatures**



Qualitative assessment


West coast
+ no sample
50
100
200
cells l-1
x no sample
10
20
30
STX eq 100 g-1
Latitude
60
59
58
57
56
55
J
F
M
A
M
J
J
A
S
O
N
D
Month

East coast


+ no sample
x no sample
300
500
1000
cells l-1
30
50
100
STX eq 100 g-1
Latitude
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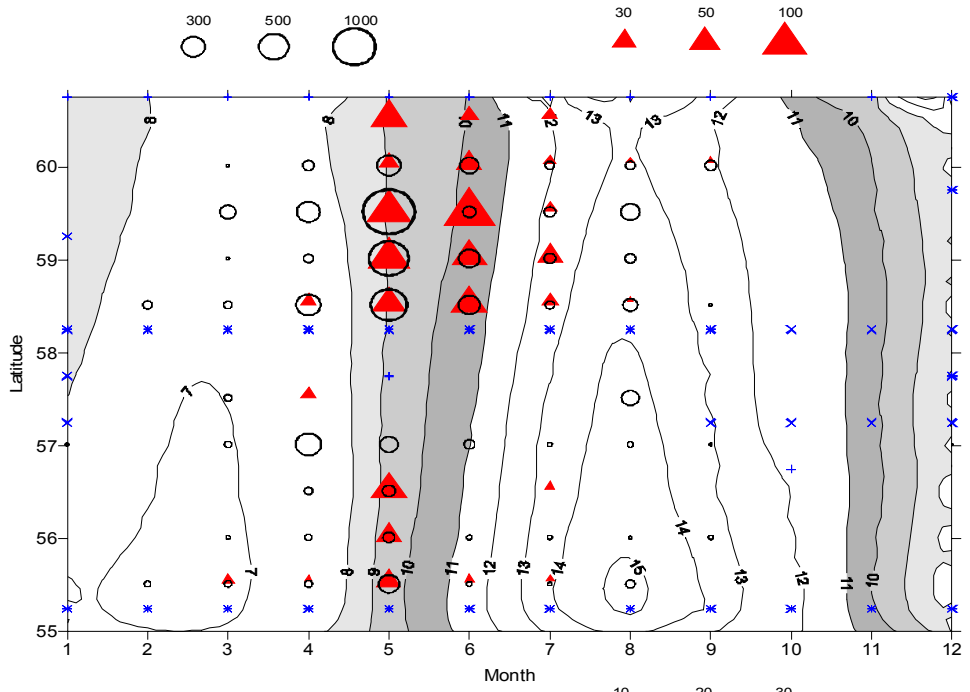
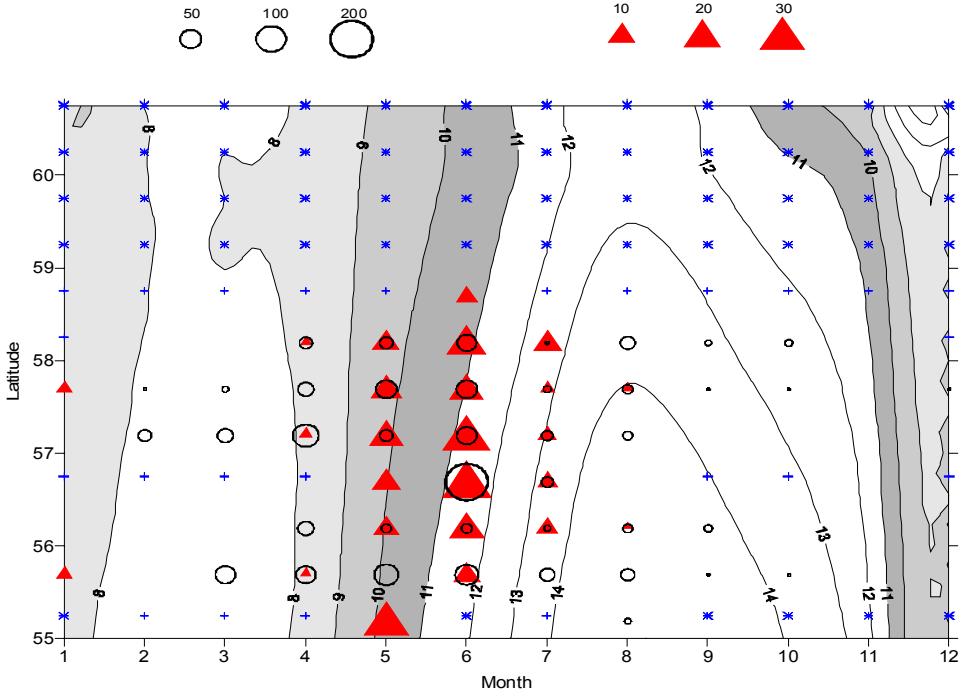
Month

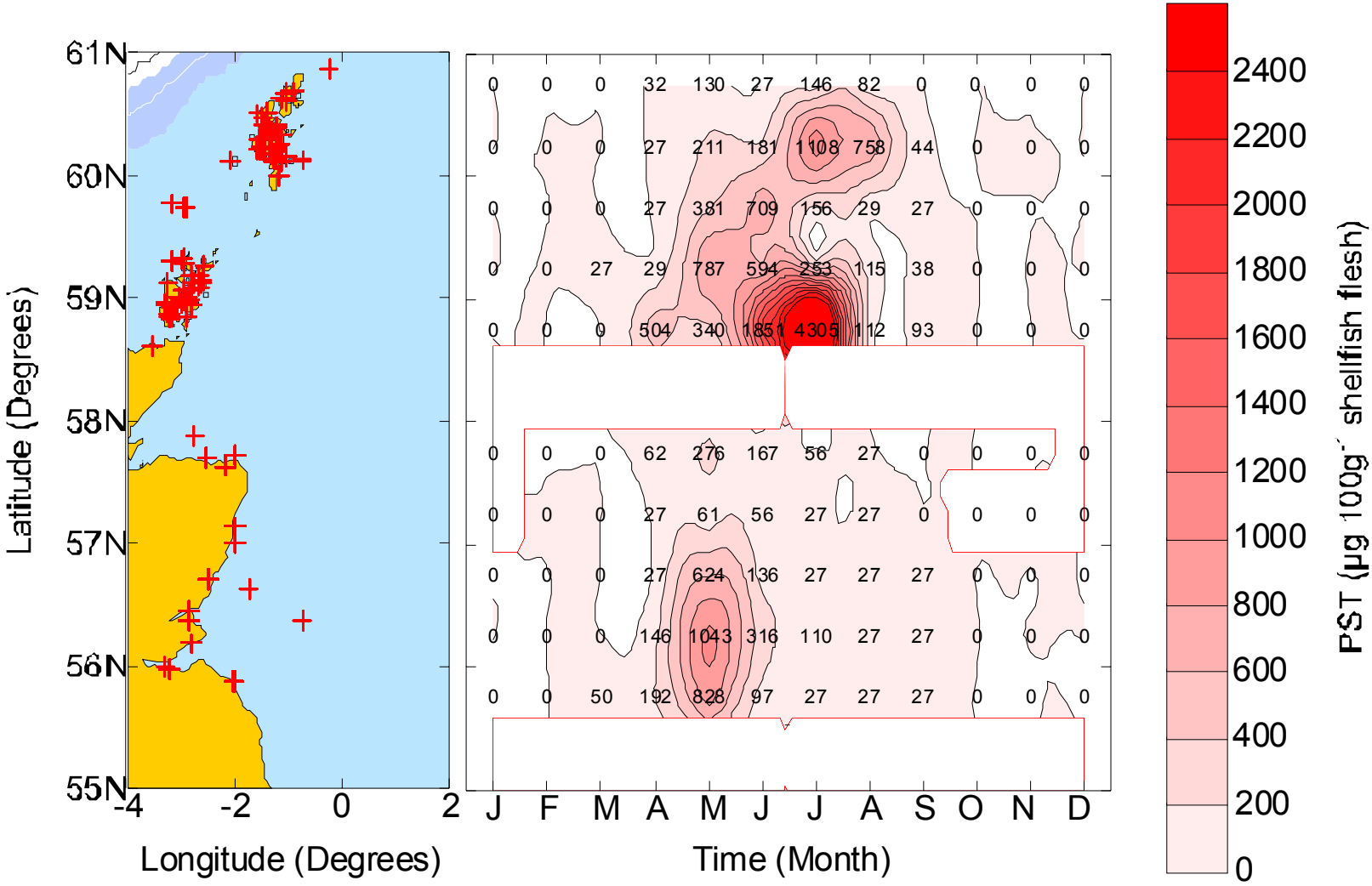
East coast



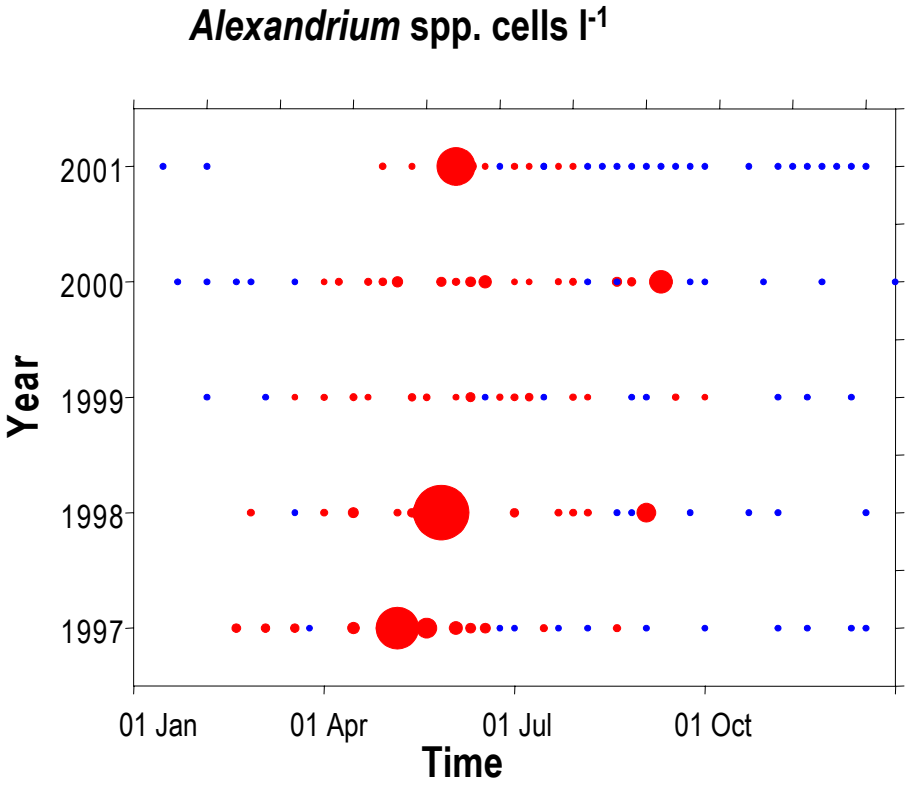
West coast

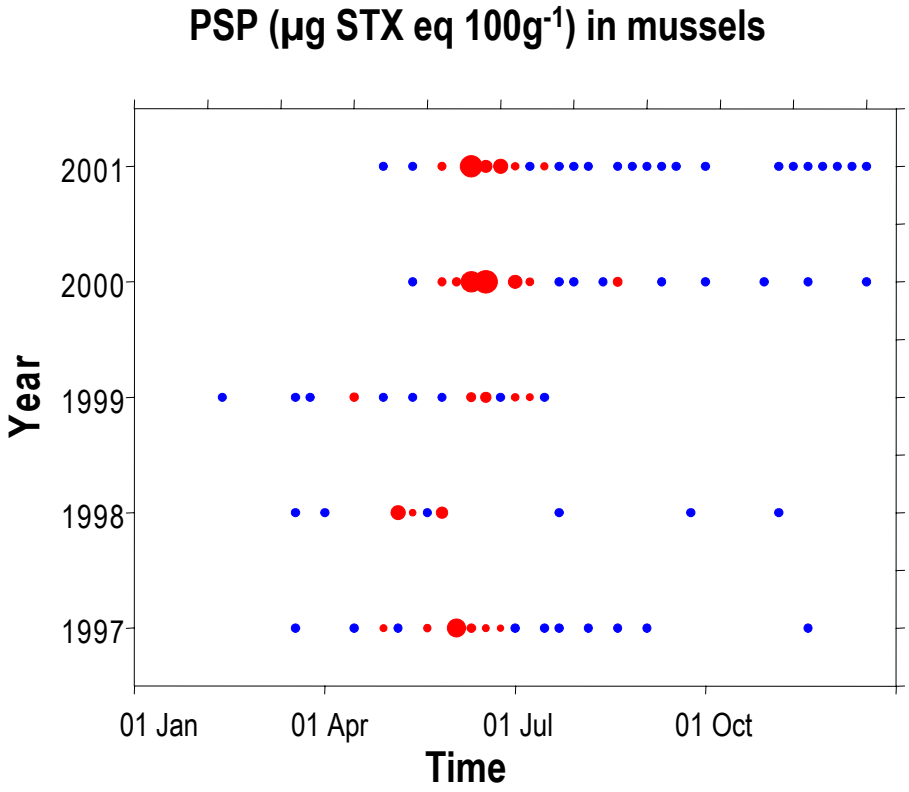


East coast


61N
60N
59N
58N
57N
56N
55N
Latitude (Degrees)
-4
-2
0
2
Longitude (Degrees)
J F M A M J J A S O N D
Time (Month)
0 0 0 32 130 27 146 82 0 0 0 0
0 0 0 27 211 181 1108 758 44 0 0 0
0 0 0 27 381 709 156 29 27 0 0 0
0 0 27 29 787 594 253 115 38 0 0 0
0 0 0 504 340 1851 4305 112 93 0 0 0
0 0 0 62 276 167 56 27 0 0 0 0
0 0 0 27 61 56 27 27 0 0 0 0
0 0 0 27 624 136 27 27 27 0 0 0
0 0 0 146 1043 316 110 27 27 0 0 0
0 0 50 192 828 97 27 27 27 0 0 0
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
PST (µg 100g⁻¹ shellfish flesh)

Scapa Bay, Orkney



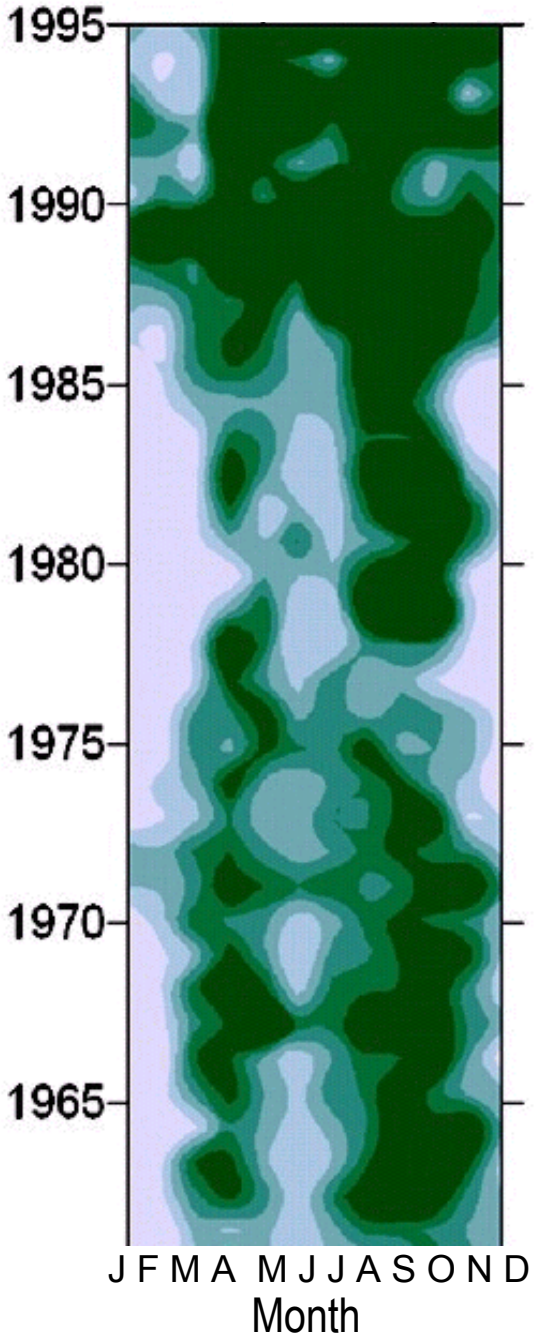


- Zero *Alexandrium* spp.
- *Alexandrium* spp.
  (values range from 20 - 5,700 cells/l)

- Zero PSP recorded
- PSP recorded
  (values range from 25 - 250 µg STX eq/100g)

North Sea
Primary Production Index

- Continuos Plankton Recorder
- Before 1985:
  - Spring Autumn blooms
- After 1985:
  - Continuos bloom
- Climate change altering plankton cycles



Reid et. al., 2001

Conclusions for monitoring programmes

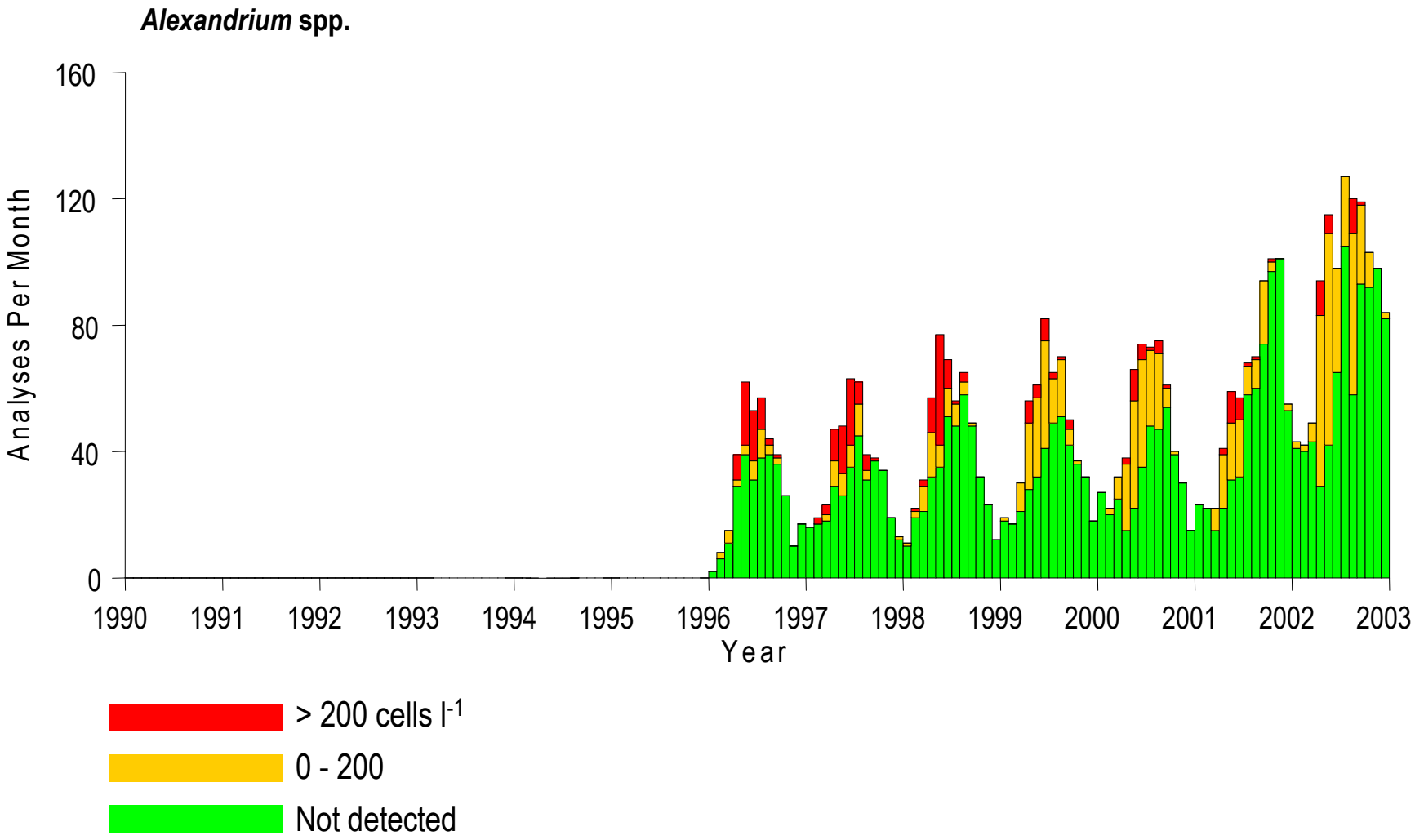
Occurrence of shellfish toxins in Scotland?

- > 10 years of monitoring data
- Periodicity of sampling is important
- Consistency of sampling (toxin-phyto-env)
- Phytoplankton composition

- General trends can be observed
- Little is known about species which are routinely present

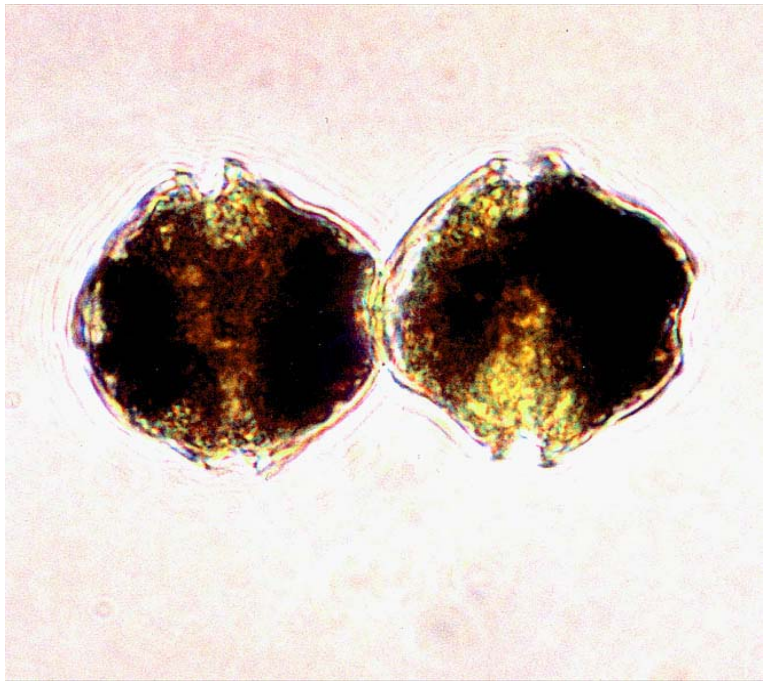
Requirement for alternative tests

Alexandrium spp. Analyses



Alexandrium spp. in Scottish Waters

- **Samples are preserved in Lugol's iodine and identified to genus level only**



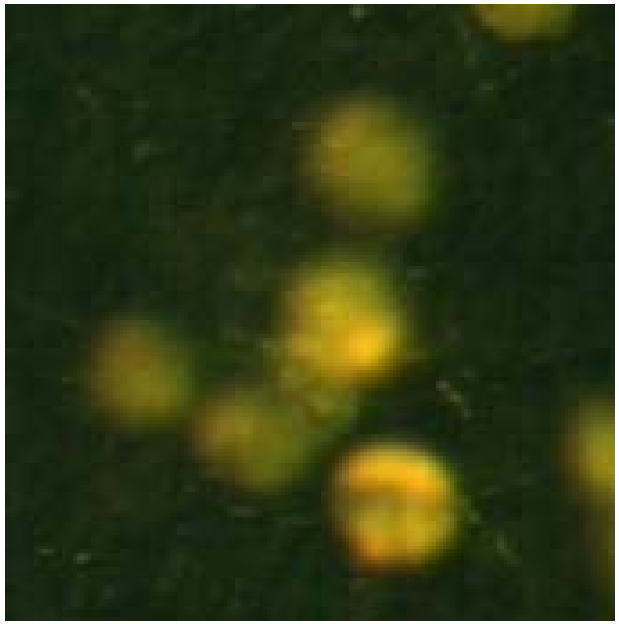
1) Shape **2) Presence/absence of pores**

Microscopy ..... Labour intensive

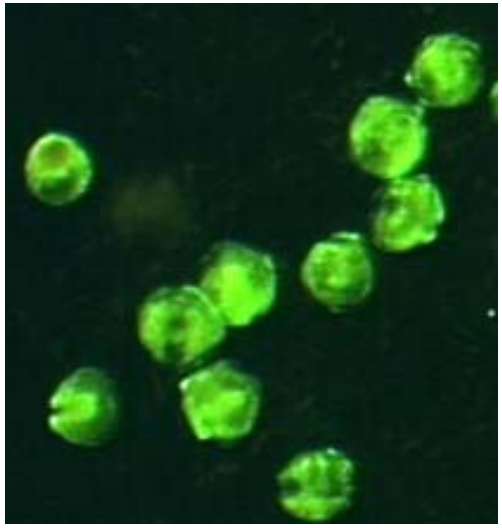
Traditional method of identifying and counting algal cells

Use of molecular probes

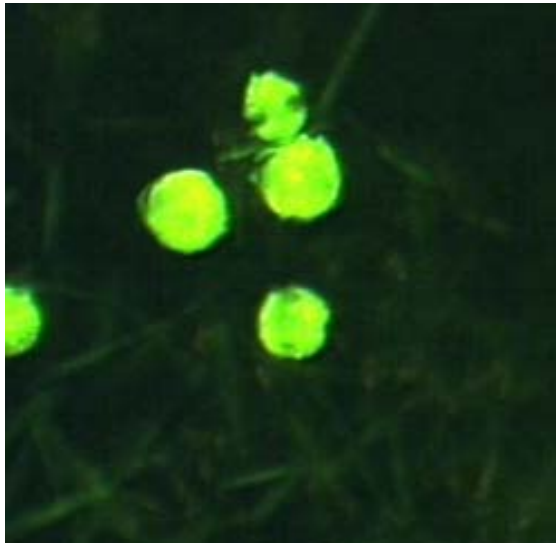
No Probe



Probe Applied

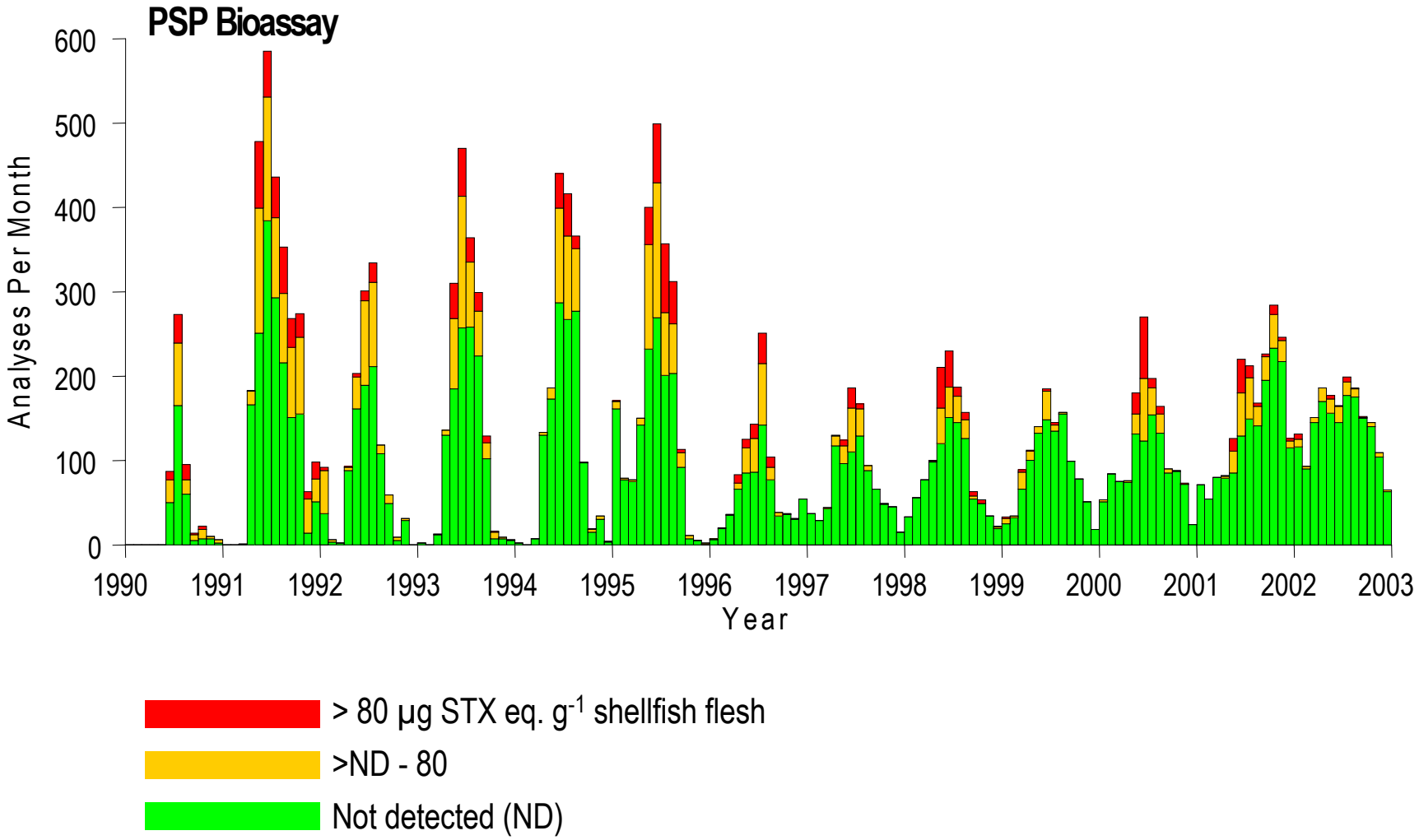


Alexandrium tamarense **(NA1)**



Alexandrium tamarense **(NA1) and** ***Pseudo-nitzschia*** **spp.**

PSP Bioassays

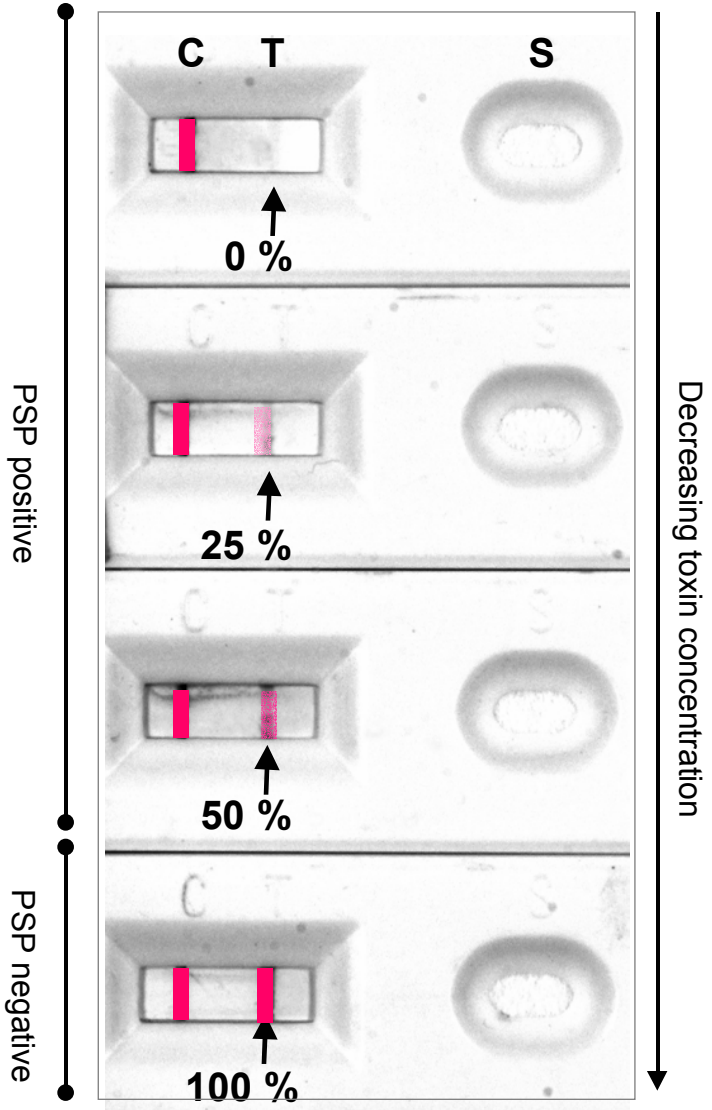


Methods for monitoring PSP Toxins

- **Standard AOAC procedure for PSP toxins analysis is the bioassay**
  - **Expensive, requires mice, not sensitive (20 % error)**

- **Various alternative methods**
  - **MNB tissue culture assay**
  - **HPLC**
  - **ELISA**
  - **MIST (Jellet Rapid tests for PSP)**
  - **Receptor binding assays**

Jellet Rapid Test

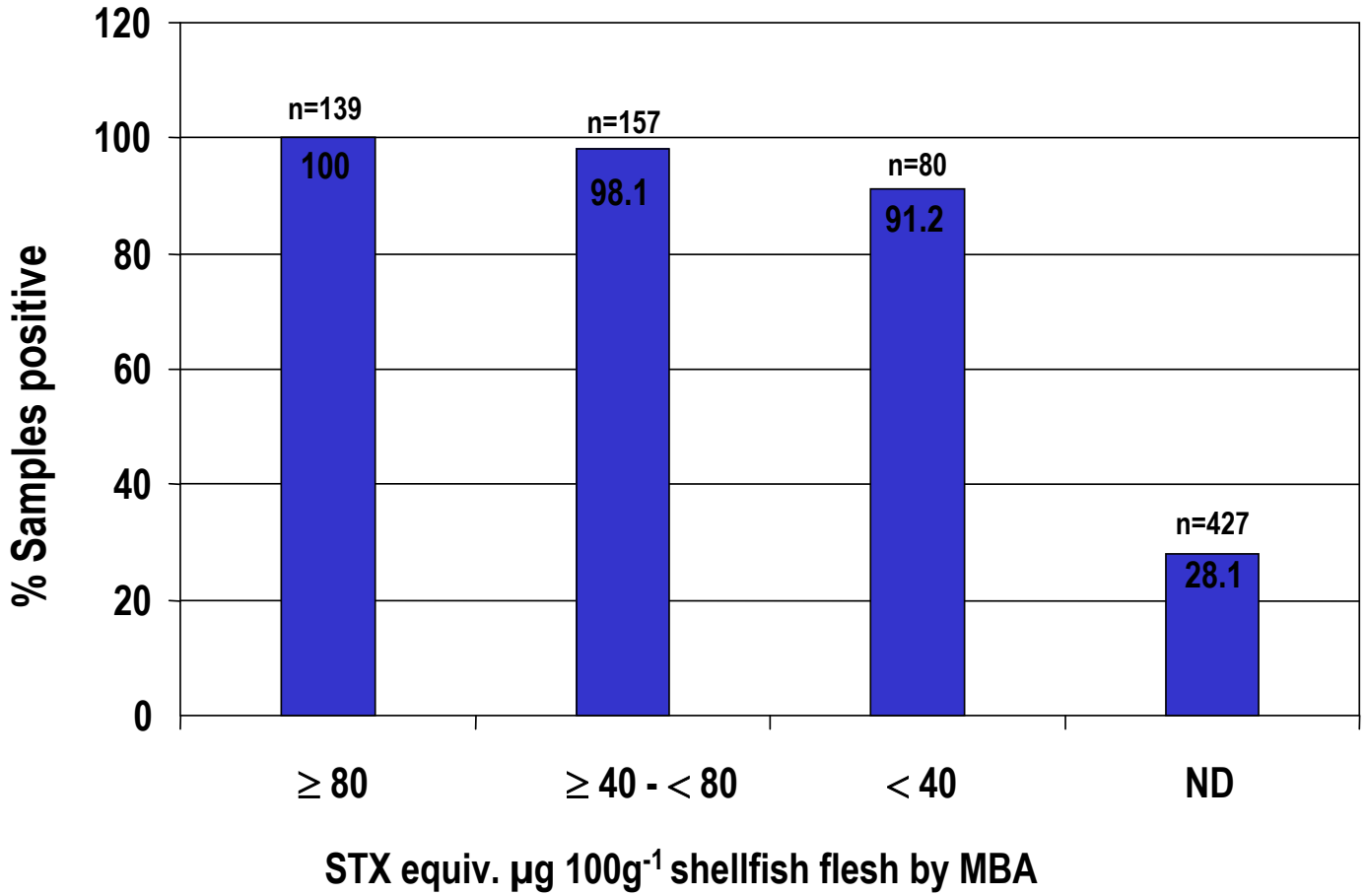


- Quick and easy
- Lateral-flow immunogromatography
- Qualitative (yes/no) indication
- Result in < 20 min
- No specialist equipment or training
- Monitoring and shellfish harvest management

Evaluation of MIST Alert™ Kit

- > 800 shellfish samples tested in 2000 / 01
  - King Scallop, *Pecten maximus*
  - Queen scallop, *Aquipecten opercularis*
  - Mussels, *Mytilus edulis*
  - Pacific Oyster, *Crassostrea gigas*
  - Northern Oyster, *Ostrea edulis*
  - Cockles, *Cerastoderma edule*
  - Razor fish, *Ensis ensis*
- All samples tested 'blind'
- Categories - Not detected, < 40, 40-80, > 80 μg^{-1} STX equiv. 100 g^{-1} shellfish flesh

Positive samples by MIST Alert™ compared to MBA



MIST Alert™ Kit

- Results show good correlation with MBA positive results
  - Journal of AOAC International, Mackintosh *et al*. 2002
  - Toxicon, Jellet *et al*. 2002
  - Journal of Shellfish Research, Mackintosh and Smith 2003

- MIST Alert™ used in monitoring to eliminate negative and low toxicity PSP toxin samples

- Use of kit could reduce number of MBAs required by 50 %

- Method for quantitative analysis required

Conclusion

- > 10 years of monitoring data (for food safety)

- Question remains:

  "What is the link between environment, climate, phytoplankton and the occurrence of shellfish toxins in Scotland?"

- Periodicity and integration of sampling is important

- Monitoring Programme for food standards and environmental issues (ie, Marine Stewardship, WFD, OSPAR, aquaculture management, etc)

Require simple, cheap, quick, reliable and accurate detection methods for phytoplankton and toxins

Acknowledgement

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Eileen Bresnan, Bill Turrell