REVIEW OF PHYTOPLANKTON MONITORING 2005

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Introduction
A national phytoplankton monitoring programme, has been in operation in Ireland since 1986, and fulfils requirements of the EU Council Directive 91/492/EEC.

This programme provides an important part of the baseline data in the overall integrated shellfish monitoring programme. The analysis of samples received on a regular basis from a site can provide very important information in assembling a population profile for the area. This helps in crucial decisions, for example in Management Cell Decisions - conducted by representatives from the industry, MI, FSAI and DCMNR - when borderline toxin results are present.

Phytoplankton monitoring is also hugely important in the Water Framework Directive, which all EU countries must follow, in developing an index of water quality in Ireland and Europe. The Irish Monitoring programme also gives valuable public health information to County Councils, Environmental Health Officer’s and the public during times of bloom events.

Overview
The following paper provides an overview of phytoplankton sampling, analysis and reporting in 2005. The occurrence of potentially toxic and harmful phytoplankton found in Irish coastal and shelf waters in 2005 is also reviewed and the quality scheme in operation is described.

Methodology

Sampling Sites
Phytoplankton sampling sites are located around the Irish coast, usually within shellfish production areas or adjacent to finfish sites. Generally, samples submitted from south-west to south-east coastal sites are analysed in the MI laboratory based in Bantry, Co. Cork, while all remaining samples submitted are analysed in the MI laboratory based in Galway.

Throughout 2005, over 2100 samples from 61 shellfish sites and 42 finfish sites around the coast were submitted to the phytoplankton laboratories. Of these, almost 92% were processed as part of the National Monitoring Programme. The remaining were analysed as part of various research projects.

Sampling Protocol
The Lund tube sampling method accounted for almost 53% of samples collected in 2005, with 26% sampled from the surface. However, almost 18% of samples received by the laboratories gave no information on sampling method. Rejected samples accounted for 4% in 2005 - a reduction from 9.9% in 2004 and 12.6% in 2003. This drop is due to a combination of improvements made to both procedures and sampling strategies.
**Sampling Analysis & Reporting**

All samples analysed for the presence of toxin producing problematic phytoplankton are examined using the Utermöhl method (Trondsen, 1995) following INAB accredited procedures. The method has a sensitivity of 40 cells l⁻¹. By the beginning of November 2005, the results of a total of over 1300 samples were reported back to the industry and related bodies, in over 246 phytoplankton reports, issued on a daily basis. The overall turnaround time from laboratory receipt to reporting is ~ 90% within one working day, and 98% in two working days, well exceeding the 80% within two working days requirement as stated in the service agreement between the MI, FSAI and DCMNR.

**Toxic phytoplankton in Irish waters in 2005**

There are four main toxic algal groups that occur in Irish waters. These are the phytoplankton species that produce the toxins that cause, Diarrhetic Shellfish Poisoning (DSP), Paralytic Shellfish Poisoning (PSP), Amnesic Shellfish Poisoning (ASP), and Azaspiracid Poisoning (AZP). In previous years closures in shellfish growing areas mainly resulted from DSP events, with localised closures in Cork Harbour due to PSP events. However, in 2005, prolonged closures also occurred due to ASP and AZP events.

Figures 1, 2 and 4 to 7, show graphs for known or suspected toxin producing phytoplankton and their associated toxin profiles from January to November 19th (week 1 to 46), 2005. The profiles illustrated are chosen from among the sites which had consistent data-sets available, between phytoplankton counts and toxin results, due to the provision of regular water and shellfish flesh samples to MI laboratories for that time period. The toxins profiled are ASP, DSP and AZA.

**Amnesic Shellfish Poisoning**

Amnesic Shellfish Poisoning (ASP) toxins (domoic acid), are produced by the diatom *Pseudo-nitzschia* spp. There are eight identified species in Irish waters – six of which are potential domoic acid producers. Amnesic Shellfish Poisoning toxicity has generally only been found in scallops in Ireland. However in 2005, mussel and a lower number of oyster closures occurred in the south west and to a lesser extent in the north west due to this toxin (Clarke *et al.*, 2006). This occurred in April to mid-May and was associated with a low biomass monospecific bloom of *Pseudo-nitzschia* spp. By the end of May however, the toxicity had fallen and all areas had re-opened. Later, in August and September there were further increases in *Pseudo-nitzschia* spp. numbers at several sites, in particular Kenmare and Bantry. Numbers peaked at 2.3 million cells/l in two sites in Galway. However no toxicity was associated with these blooms.

Due to the limitations of light microscopy (Figure 3) in identifying minute, detailed morphological structures found in the *Pseudo-nitzschia* genus, cells are generally categorised into one of two groups. These are *Pseudo-nitzschia seriata* group – which contains the main toxin producing species, and *Pseudo-nitzschia delicatissima* group. Work in identifying the possible causative species for the April toxicity is continuing.

Figures 1 and 2 show this correlation between cell numbers of the two main *Pseudo-nitzschia* groups described above, and toxicity in the sites shown, for the toxic event early in the year, and the non-toxic bloom later in the year. There were differences in the toxicity of the individual blooms, most likely due to different species succession through the year.
**Figures 1 and 2.** Cell counts of *Pseudo-nitzschia seriata* group and *Pseudo-nitzschia delicatissma* group from North Chapel and Snave in Bantry North, for week 1 to 46, 2005. It also shows the toxin levels of ASP for the same time period.

**Figure 3.** *Pseudo-nitzschia* spp. cell showing the level of detail required to identify it to species level, using electron microscopy.
Paralytic Shellfish Poisoning
Paralytic Shellfish Poisoning (PSP) toxins - saxitoxins - are produced by *Alexandrium* spp. Due to the potential severity of the toxin, the presence of this species in water samples triggers increased testing of shellfish samples for PSP toxins.

To date the main production area that has experienced closures due to PSP toxins is North Channel in Cork Harbour. Levels of *Alexandrium* spp. were generally observed at low levels all around the Irish coast throughout May to August (when compared to 2004), with the highest levels observed in North Channel, Kinsale, Oysterhaven, Loughras Beg & Greenore. Paralytic Shellfish Poisoning toxicity occurred in mussels in early to mid June in North Channel, Cork Harbour (Clarke *et al.* 2006), which corresponded a rise in *Alexandrium* spp. levels to 1080 cells/litre. Numbers subsequently decreased and rose again to 1640 cells/litre in early July. However, no toxicity was present at that time. The levels and distribution of *Alexandrium* spp. were observed to decrease further around the coast in September and were observed to be mainly confined to the south and south west at low levels in October.

Diarrhetic Shellfish Poisoning
Diarrhetic Shellfish Poisoning (DSP) toxins (okadaic acid and DTX’s) are produced by the dinoflagellates *Dinophysis* spp. and *Prorocentrum lima*. The majority of closures in Irish production areas occur as a result of this toxin. Toxicity in shellfish can be recorded at very low cell counts (>200cells/l).

During May, in the majority of west and southwestern locations, *Dinophysis* spp. were present at typically low levels. In June, levels were observed to increase in a small number of locations in the south west, but typically remained at the same levels as those observed in May. In the west and north west significantly large increases in cell numbers were observed, but generally decreased in July and decreased further in August.

This decrease was not observed in the south west, and significant cell counts occurred in all sites in August. In September, cell counts of *Dinophysis* spp. decreased rapidly in all localities - especially the south west. From October to date, no *Dinophysis* spp. have been observed.
Figures 4 and 5. Cell counts of *D. acuminata* and *D. acuta* from Snave in Bantry North, and Tahilla in Kenmare Bay, for week 1 to 46, 2005. These figures also show the toxin levels of Total Okadaic Acid equivalents for the same time period.

**Azaspiracid**

Azaspiracid (AZA) toxins, have been associated with the dinoflagellate *Protoperidinium crassipes*. In other genera as the production of toxins can come from several species, it may be the case therefore that more than one *Protoperidinium* species may produce AZA.

The cell counts and AZA levels are shown in the figures 6 and 7 and no apparent correlation was evident between the presence of *Protoperidinium* spp. in the water and the toxin level increases, which occurred. In all sites *P. crassipes* was present at levels averaging 40-80 cells/litre.
Figures 6 and 7. Cell counts of *Protoperidinium* spp. from Snaive in Bantry North, and Ardgroom in Kenmare Bay, for week 1 to 46, 2005. It also shows the toxin levels of AZA for the same time period.

**Other problematic species in Irish waters in 2005**

Two main problematic species occurred in Irish water’s during 2005 at bloom levels. *Noctiluca scintillans* occurs annually during the summer/early autumn months. In 2005 the highest concentration recorded was in Donegal Bay on 5th September at 7.2 million cells/litre, and Kilmakillogue, Co. Kerry on 4th August at 7 million cells/litre. No human health problems were associated with these blooms. A second more widespread and damaging bloom of *Karenia mikimotoi* also occurred during the summer months of 2005. A detailed report of this bloom and its impact on coastal communities is given in these proceedings and in Silke *et al.*, 2006.
Quality System
Towards the end of 2004, the phytoplankton laboratories of MI applied to the Irish National Accreditation Board (INAB) for ISO 17025 accreditation, in the test method used for the identification and enumeration of toxic and problematic species, using the Utermöhl method of analysis.

In March 2005, this award was presented to both the Galway and Bantry laboratories. The MI is the first national phytoplankton monitoring laboratory in Europe to receive accreditation for this analysis and had to meet the quality assurance and procedural guidelines under ISO 17025. One of the requirements included an audit trail for species identification, which was satisfied by introducing a system of recording a series of photo-micrographs for each species observed by an analyst over specified time period.

Irish National Accreditation Board accreditation ensures a high standard certification process, guaranteeing traceability, confidence and consistency in all samples analysed.

References