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

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

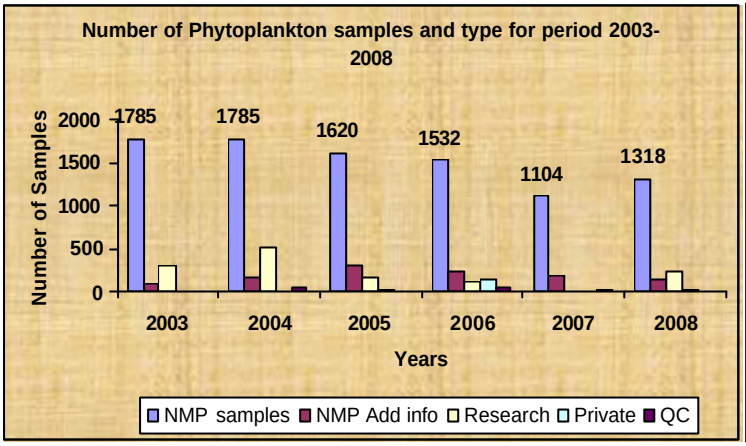
A brief introduction and background of the Phytoplankton National monitoring programme was given to the audience. The programme which is underpinned by the EU directive 854/2004, a Europe wide legislation states that shellfish producing areas have to be monitored for the presence of toxic algae. This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

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

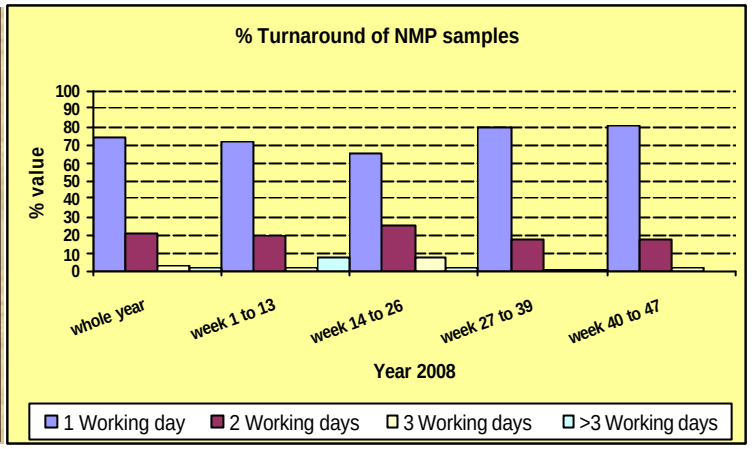
The National Monitoring programme for phytoplankton is a well established programme and this was shown through the improvement and refinement of Phytoplankton shellfish and finfish sites around the country. One important development in the last 2 years has been to increase the number of sentinel sites.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

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

Graph 1

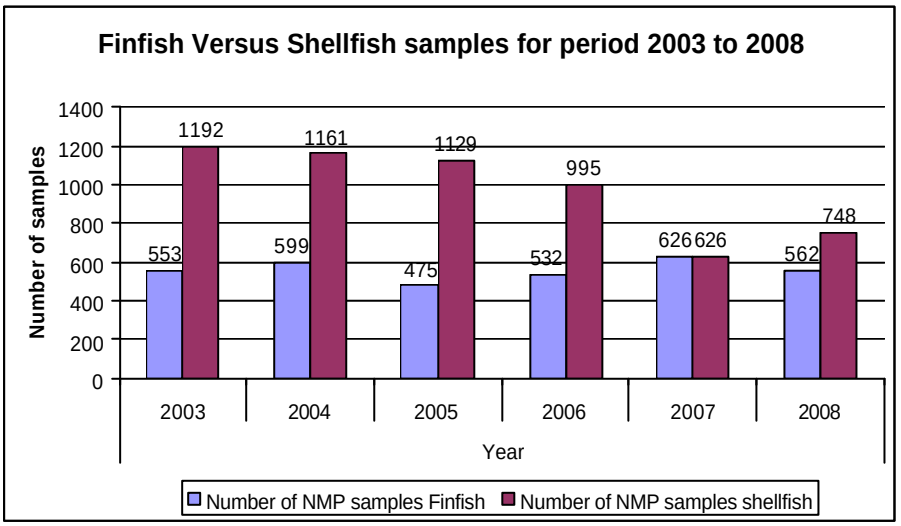


Graph 2



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

Graph 3



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

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

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

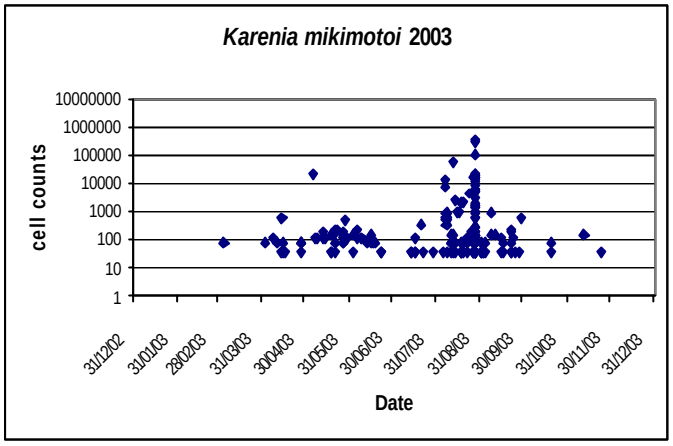
This year for the first time there were 17 labs and 37 analysts across Europe participating in the exercise. Countries like Germany, Holland or Spain are already represented together with Great Britain, Northern Ireland and Ireland.

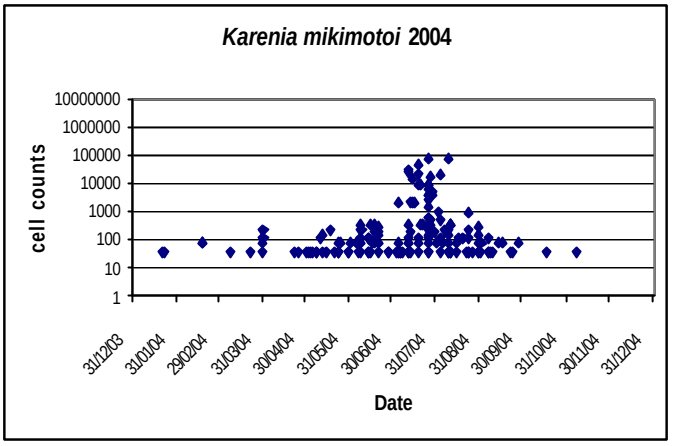
Also, we thought interesting to review the most important HABs species in Irish waters as a way to demystify the belief that HABs only occur in particularly bad years or that are rare events. To prove this theory we embarked on the reviewing of a number of HABs species over a period of 6 years, between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

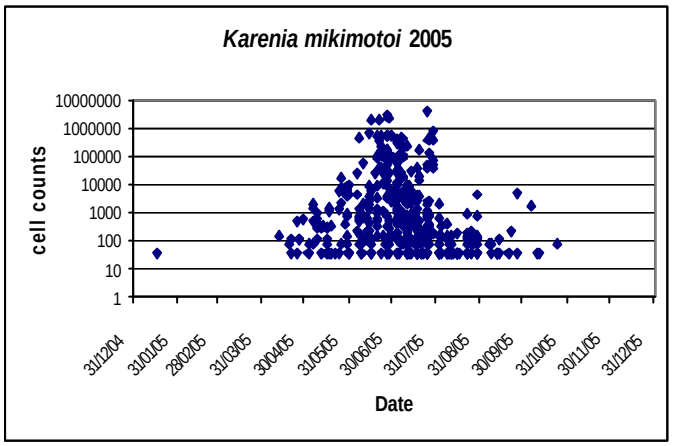
There is clear evidence from Graph 4 using *Karenia mikimotoi* cell counts in logarithmic form as an example that these species are found regularly around the Irish coast year on year that they bloom at some stage and then die down. It is certain that they not always cause harm and that the concentration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

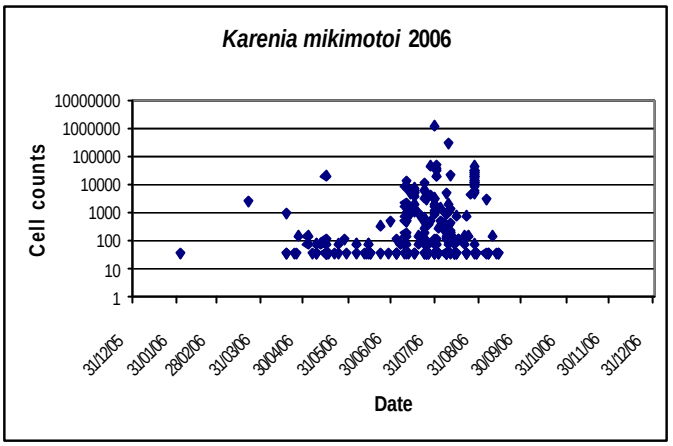
What we know now is that if you find concentrations as high as those found in 2005 for *Karenia mikimotoi* that problems may occur.

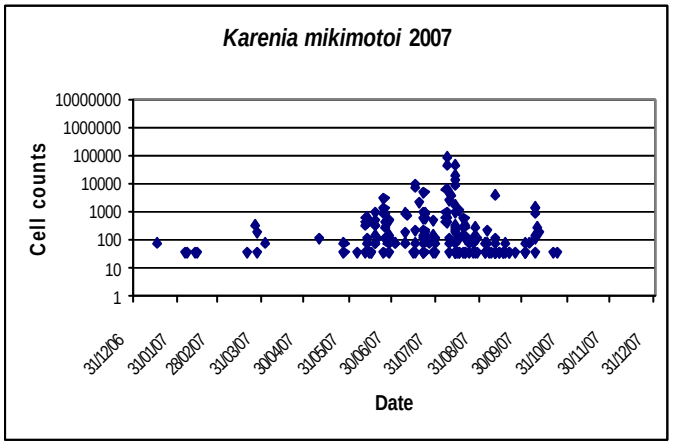
Graph 4: *Karenia mikimotoi* cell counts from 2003 to 2008

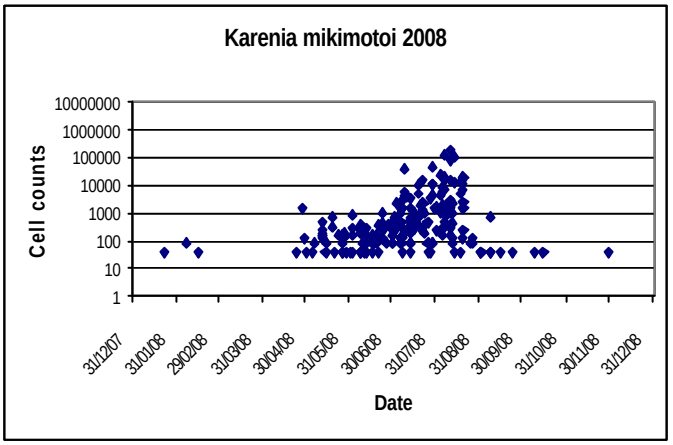












Azaspiracids update

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

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

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

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

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

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

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

Molecular tools in the Phytoplankton lab

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

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

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

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

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

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

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

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