REVIEW OF PHYTOPLANKTON MONITORING PROGRAMME AND RESEARCH ACTIVITIES

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This paper provides a review of the activities of the Phytoplankton Unit in the Marine Institute as part of the National Monitoring Programme for 2007 and compares the findings with those recorded during 2005 and 2006., It also presents an overview of the research activities carried out by the phytoplankton team during the year with a focus on culturing phytoplankton and the introduction of real time PCR techniques for phytoplankton identification. .

The National Monitoring Programme (NMP) for phytoplankton is an important element of the National Biotoxin Monitoring Programme in Ireland. The phytoplankton monitoring programme provides data and information on the distribution and occurrence of toxic and harmful algae around the coast of Ireland. The data are provided to the aquaculture industry, regulatory agencies, the scientific community and the general public. It provides key data for use in Management Cell decisions (see the paper by Devilly *et al*. in this volume). Additionally the data series will be used in other research programmes e.g. Climate Change and in fulfilling Ireland's obligations under the Water Framework Directive.

Phytoplankton species and related toxins

As shown in Figures 1 and 2 the *Dinophysis acuminata* cell numbers recorded in 2006 and 2007 were significantly lower than those recorded in 2005.

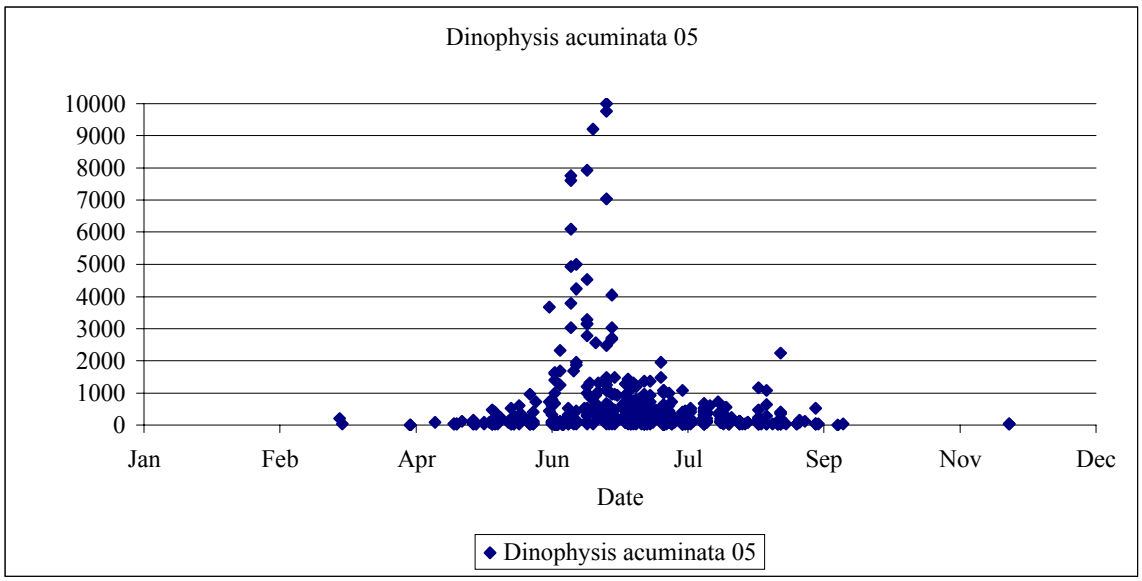

Figure 1. *Dinophysis acuminata* 2005

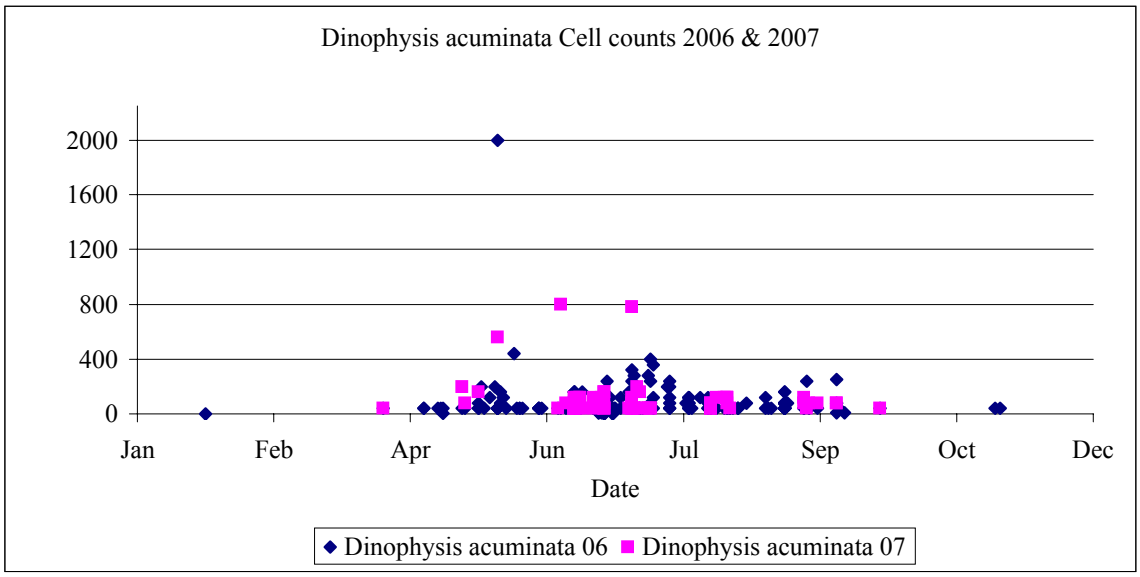

Figure 2. *Dinophysis acuminata* 2006 and 2007

The pennate diatom *Pseudo-nitzschia* spp. is known to produce Domoic acid, the primary toxin causing Amnesic Shellfish Poisoning. Figures 3 & 4 show the cell counts of *Pseudo-nitzschia* spp. for the last 3 years. . *Pseudo-nitzschia* spp. for practical reasons has been separated into two groups: the seriata group and the delicatissima group. Species from both groups can actually be toxic, but they are very difficult to identify to species level when using conventional light microscopy, so a pragmatic approach has been used to narrow down the species by grouping them in this manner.

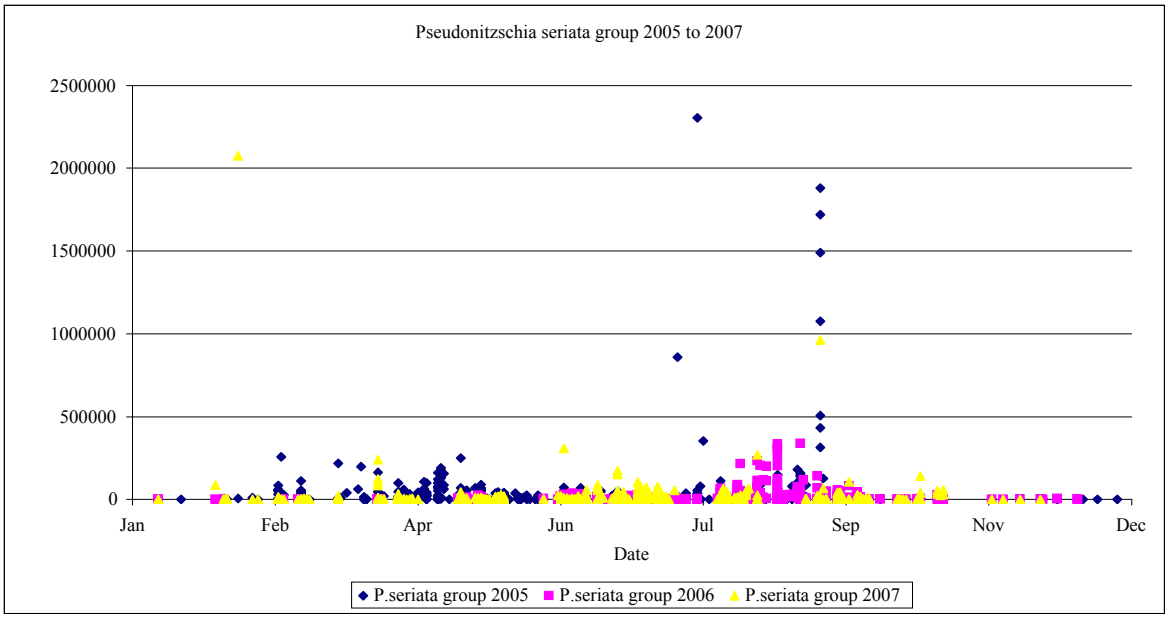

Figure 3. *P. seriata* cell counts 2005, 2006 & 2007

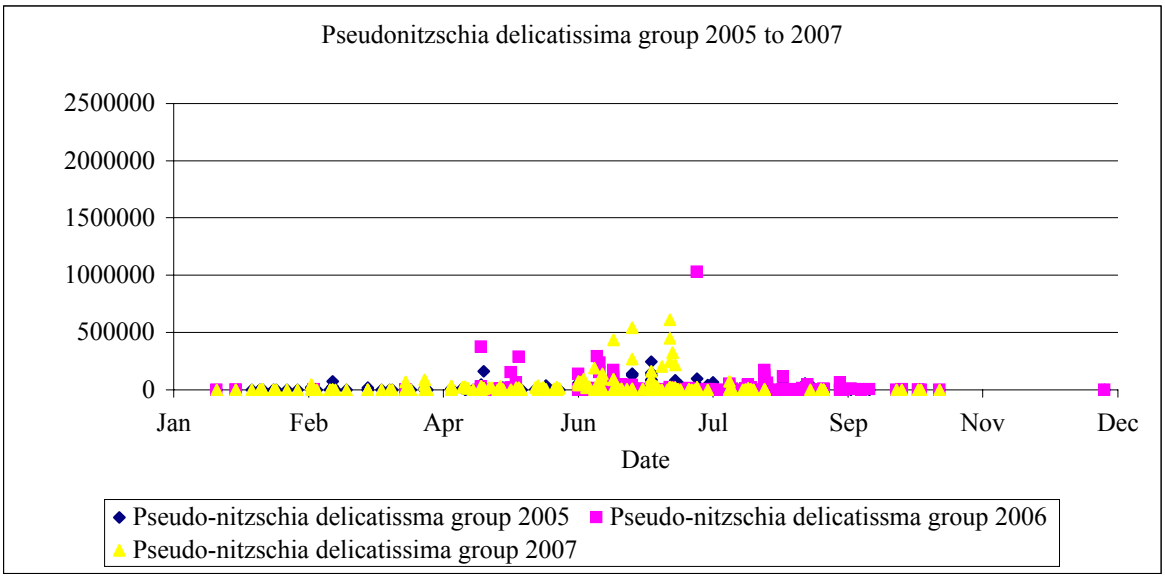

Figure 4. *P.delicatissima* group cell counts 2005, 2006 & 2007

The data presented shows that the density of these species over the last 3 years has not changed significantly. It shows that these species are very cosmopolitan and conspicuous, occurring throughout the year but in greater numbers typically during the spring bloom in April and during the summer months. However in 2005 the levels of Domoic acid in mussels for the SW coast exceeded the regulatory limit in early April (Clarke *et al.,* 2005) but this did not occur in either 2006 or 2007. This indicates that cell numbers of *Pseudo-nitzschia* spp. alone are not a good indicator of the onset of ASP toxicity and that it essential to identify the organism to species level in order to better evaluate the risk. To this end the Phytoplankton Unit is developing gene probes coupled with Real time PCR techniques to enable such data to be collected on a routine basis. (See the paper by Kavanagh *et al* this volume).

In Ireland the occurrence of PSP toxins in shellfish is mainly confined to PSP in to the Cork harbour area, where shellfish, principally mussels, become toxic usually for a short period of 1 -2 weeks in June . In 2007 PSP toxins were detected in mussels from Cork Harbour in late June and early July. The levels of *Alexandrium* cells in the water were quite low compared to 2006 (Figures 5 and 6).

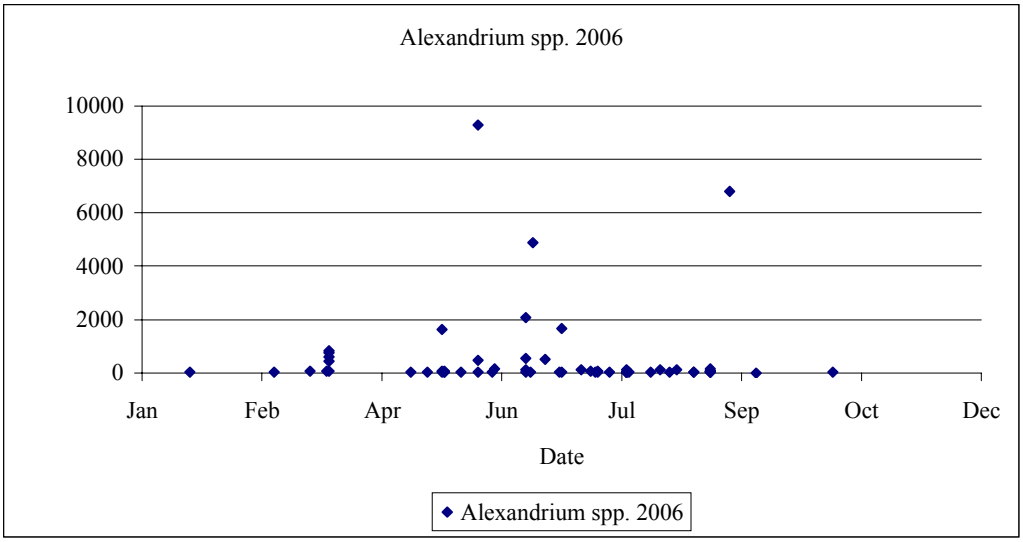

Figure 5. *Alexandrium* spp. cell counts in 2006

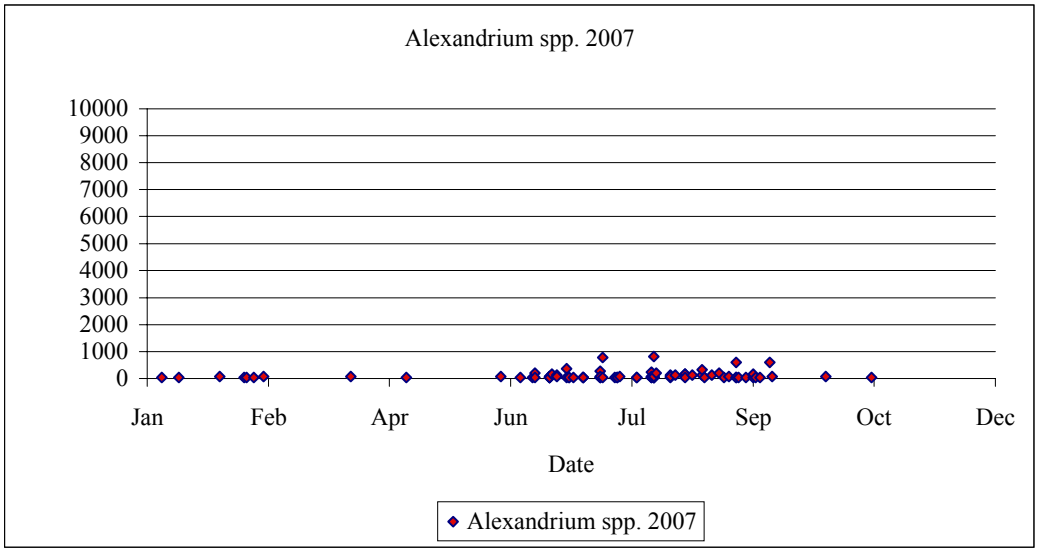

Figure 6. *Alexandrium spp.* cell counts in 2007

Azaspiracid (AZA) was first discovered in 1995 and *Protoperidinium crassipes,* taken from plankton net hauls off the SW coast of Ireland, has been reported to be the causative organism (James *et al* 2004). However, *Protoperidinium* spp. are heterotrophic organisms and could therefore accumulate the toxin through feeding upon the true progenitor, which could explain the poor correlation between the levels of *Protoperidinium* spp and the levels of AZAs detected in shellfish. Figures 7 & 8 show that *Protoperidinium* spp. are cosmopolitan but ubiquitous in Ireland, most times occurring in low cell numbers all throughout the year. In 2007 AZA was detected in shellfish at levels above the regulatory limit in October and November but no clear link is apparent between this AZA event and the cell numbers of *Protoperidinium* spp recorded at the same time.

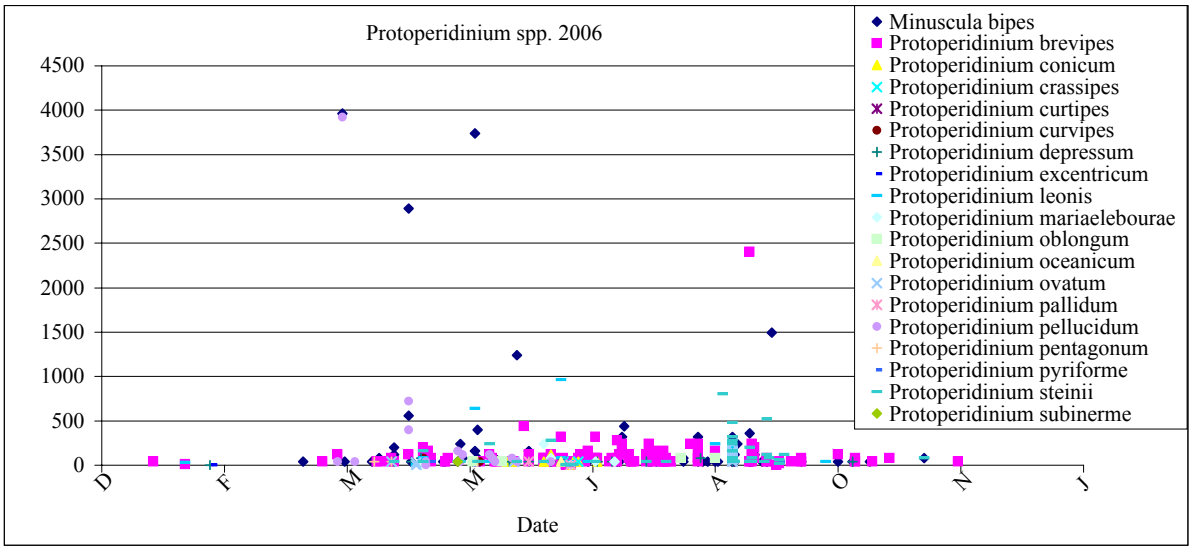

Figure 7. *Protoperidinium spp*. 2006

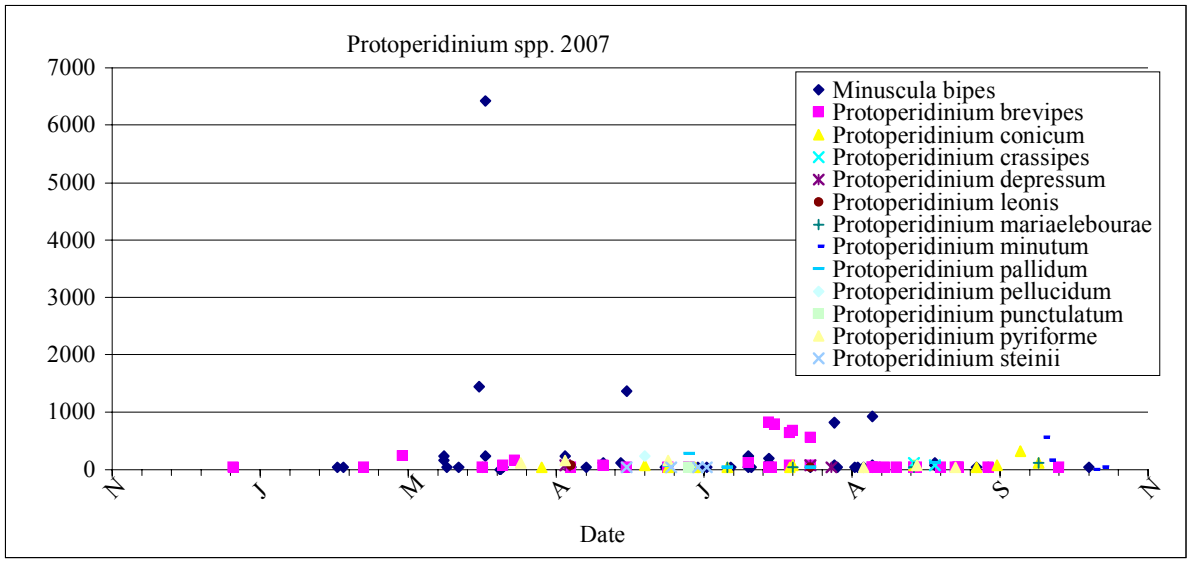

Figure 8. *Protoperidinium spp.* 2007

Temperature monitoring

The Marine Institute maintains a network of temperature probes (TidBits ™) at 11 aquaculture sites around the coast.. Each site has several sensors attached to nets or buoys at different depths and they measure temperatures hourly over a period of several months before the data needs to be downloaded. This data is a comprehensive time series of temperature data around the coast of Ireland, which can be accessed through our website www.marine.ie . Figure 9 shows an example of temperature time series for the past 4 years from Killary Harbour, County Galway. In 2007 water temperatures were generally lower than in previous years and only exceeded 14°C for a shorter period compared with the 2004 – 2006.

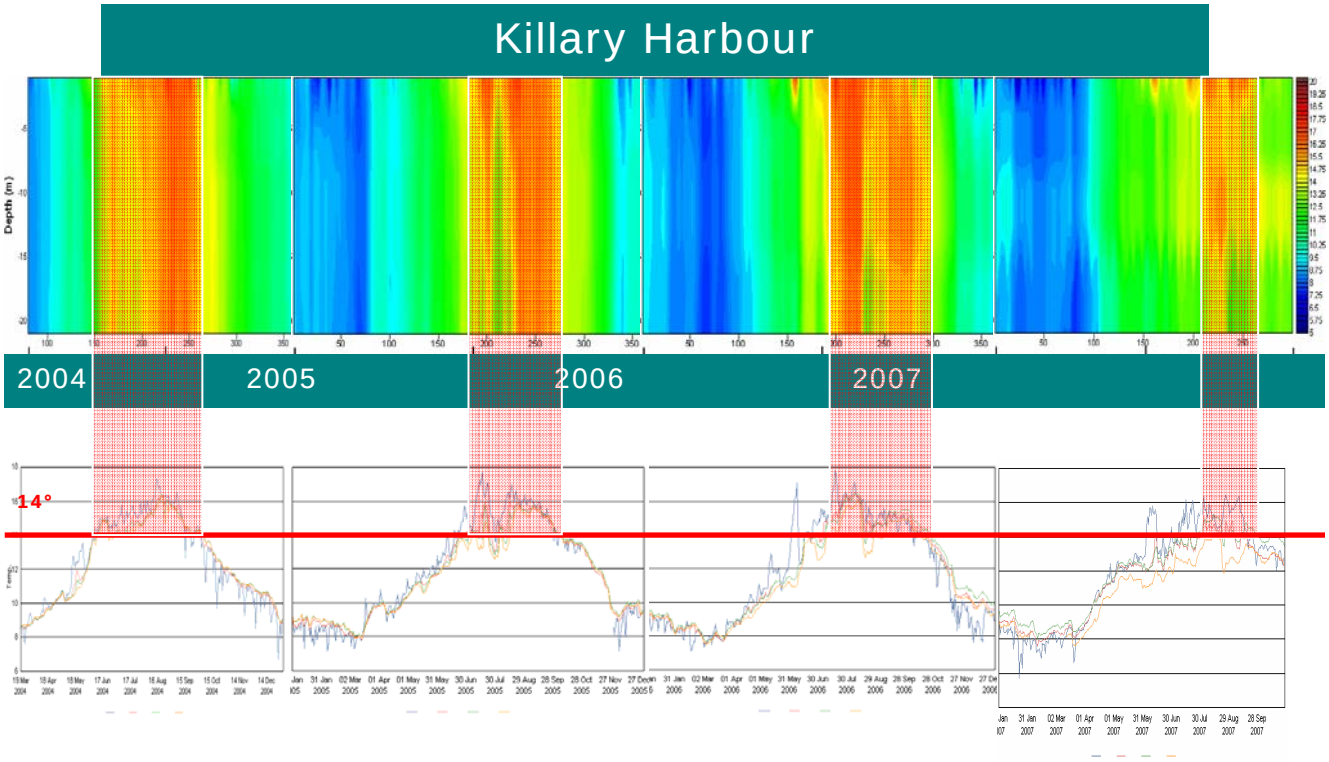

Figure 9. Killary Harbour temperature data since 2004.

Research Activities

The Marine Institute's Phytoplankton Unit has been involved over the years and continues its involvement in number of collaborative research projects at an international and national level. In the past we have been working closely in projects including BOHAB, MATSIS, METRIC and others. At present we are collaborating in a project called PHYTOTEST which involves the development of gene probes to assist in the identification of toxic/harmful marine phytoplankton species. Details of this project are presented in the paper by Kavanagh *et al* in this volume

As well as working in this project, the phytoplankton unit staff since the move to the new Headquarters at Rinville, Oranmore, County Galway, have been working and developing their skills in the new culture unit for phytoplankton. The new facilities include a walk-in incubator, several stand alone incubators, a laminar flow hood and a dedicated and fully operational culture lab (Plate 1)

Plate 1. MI Phytoplankton Culture facilities

A significant effort has been made over the last 2 years to culture ecologically important marine phytoplankton species. Some 30 strains of mostly toxic and harmful phytoplankton species have been established and used for a number of research projects. The cultures have been used in morphological studies, life history studies, toxicological studies, molecular studies but also for teaching and demonstration purposes.

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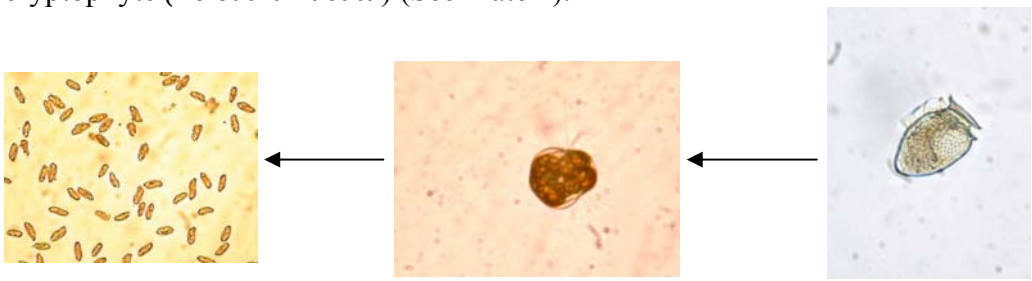
At present the culture unit is attempting to culture *Dinophysis* spp. Attempts at culturing *Dinophysis* spp. had failed over the years but in 2006 a Korean research group successfully cultured this species for the first time (Park *et al.,* 2005) The process involves feeding *Dinophysis* with a ciliate (*Myrionecta rubra*), this ciliate in turn would have to be fed with the cryptophyte *(Teleaulax acuta*) (See Plate 2).

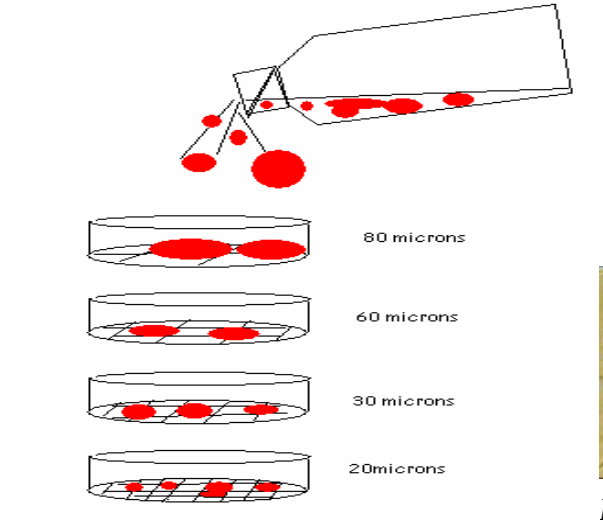


Teleaulax amphioexa *Myrionecta rubra* *D. acuminata*

Plate 2. Pictures of organisms involved in the culture of *Dinophysis acuminata*

In collaboration with colleagues D. Kulis and D. Anderson from the Woods Hole Oceanographic Institute work is ongoing to culture *D. acuta* and *D. tripos D. acuta* has been maintained in culture for the past 5 months and feeding and division has been recorded. The small cells of *D. acuta* are very similar to *Dinophysis dens* cells and it is possible that they are the same species at different stages of their life cycle. In addition we have a 3 month old culture of *D. tripos* and we have also observed feeding and reproduction giving way to small cells as has been observed with *D. acuta. It is likely that, in the near future,* the genus *Dinophysis* will have to be taxonomically completely revised.

The phytoplankton lab is also trying to isolate and culture organisms that produce Azaspiracid. This is done by obtaining live samples from AZA affected areas and carrying out fractionation of the sample to the smallest mesh possible (1µm). Cultures of the fractions are bulked up and analysed using advanced LCMS techniques (Figure 10).



Protoperidinium crassipes

Figure 10. Fractionation of live samples through mesh.

As well as culturing phytoplankton, the phytoplankton unit is part of the molecular biology facility in the MI. This unit was established recently, this year a real time PCRinstrument has been commissioned and is functioning at present. Phytoplankton personnel have started training in molecular techniques and our aim is to use these molecular tools to identify toxic phytoplankton found in the Irish coastal waters, create a database of Irish strains and ultimately develop gene probes as a risk management tool for the phytoplankton monitoring programme.

References

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