**Marine Biotoxins – Research**

**Introduction**
The general question of what causes some phytoplankton to produce marine biotoxins and what determines the frequency of toxic algal events is the subject of worldwide research. Fisheries Research Services (FRS), in collaboration with other laboratories, has a programme to develop methods for identifying toxic phytoplankton and detecting marine biotoxins. FRS also plays an important role in the research into these toxins, bringing together its experience in hydrography, chemistry and biology.

**Phytoplankton Detection**
Identifying organisms to species level is necessary because not all species of phytoplankton are toxic.

The identification of phytoplankton which produce biotoxins to species level is an extremely time consuming and specialised task, requiring expensive electron microscopy techniques.

FRS has an on-going monitoring programme for toxin producing phytoplankton, which has been in place since 1995. Toxic phytoplankton species that are problematic to the Scottish shellfish industry include *Pseudo-nitzschia*, *Alexandrium* and *Dinophysis* species.

The use of specific fluorochrome-labelled DNA probes and fluorescence microscopy is a fast alternative to these techniques. Developments in this technology have shown that unique parts of the DNA of phytoplankton can be used to identify them to species level.

To date, there is limited information on the genetic diversity of Scottish strains of phytoplankton. DNA probes have been designed for a number of different species in the USA. Some studies have shown that probes for some of these species can be used with European strains. However, regional variation in the genetic makeup of some phytoplankton means it is critical to ‘tune’ DNA probes to suit the geographical region of interest. Researchers at FRS are examining the genetic diversity of Scottish phytoplankton and are developing methods to use DNA probes in future monitoring programmes.

**Bacteria in Toxin Production**
The occurrence of PSP toxins in shellfish is generally attributed to their filter-feeding on certain species of dinoflagellates (*Alexandrium*) that are associated with PSP toxin production. However, controversy surrounds suggestions that bacteria are also capable of producing marine biotoxins, either alone or in conjunction with dinoflagellates.

Bacterial involvement in marine biotoxin production was first proposed in the early 1960s, due primarily to observations of ‘bacteria-like’ structures within dinoflagellate cells. In an EU funded project co-ordinated by FRS fluorescence microscopy, and DNA probes designed from sequence data of bacteria isolated from dinoflagellate cultures, have been used to detect bacteria attached to *Alexandrium* cells. The use of transmission electron microscopy (TEM) and confocal laser scanning microscopy (CLSM) confirmed the presence of bacteria inside some dinoflagellates.
Researchers at FRS have provided evidence that these bacteria can influence the type and concentration of toxins produced by *Alexandrium* species. Some of this information is being used to study the genetics of toxin production, which could lead to the development of improved tests for their detection.

**Toxin Detection**

Testing of shellfish samples for paralytic shellfish poisons (PSP) at FRS began in 1990. The main tool for monitoring PSP in shellfish has been a biological assay. This assay is costly and requires skilled laboratory personnel. Hence, aquaculturists and fishermen are unable to use the assay in shellfish harvest management, and shellfish processors do not have a readily available end-product test.

Canadian scientists have developed a commercially available antibody-based test kit called the Jellet Rapid Test for PSP, which provides a qualitative (yes/no) indication of the presence of PSP toxins in less than 20 minutes.

In a project commissioned by the Food Standards Agency, FRS has conducted a series of trials, where results generated from the Jellet Rapid Test have been compared to the biological assay. Further field trials were carried out during which shellfish farmers, and processors evaluated the ease of use and interpretation of results obtained using the kit. Results from these trials suggest that the Jellet Rapid Test accurately detects the presence of PSP toxins in shellfish, has the potential to reduce the requirement for biological assays in PSP toxin monitoring and could be used as a shellfish management tool and end-product test.

Chemical methods are also applied to toxin detection. High Performance Liquid Chromatography (HPLC) is routinely used for monitoring amnesic shellfish poisons (ASP). A more complex technique, Liquid Chromatography-Mass Spectrometry (LC-MS), is being developed, at FRS, for the detection of diarrhetic and lipophilic shellfish poisons (DSP, LSP), which gives more precise information on chemical constituents of the toxin groups.

**Toxin Metabolism**

Rates of accumulation and depuration of marine biotoxins vary greatly amongst different shellfish species. For example, mussels (*Mytilus edulis*) expel toxins within a short time whereas, the king scallop (*Pecten maximus*) can remain toxic for many months.

Little is known about the metabolism of toxins in fish and shellfish, although the involvement of detoxifying enzymes has been suggested. These enzymes are well known in the breakdown of toxic chemicals, in mammals, birds and plants. Bacteria may be involved in toxin depuration in shellfish, as they are known to biodegrade chemicals using a variety of mechanisms, which in some occasions, involves similar enzymes to those found in mammals.

Research at FRS has demonstrated that some detoxifying enzymes are induced in scallops and salmon by exposure to PSP. Additionally, recent data suggest that some marine bacteria break down this group of toxins.

This research is continuing with the eventual aim of providing information that may be used to improve our management strategies for marine biotoxins.