Toxic Phytoplankton in Irish Waters

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Introduction:

A brief review of Toxic Phytoplankton followed by some current FRC studies:

The subject of harmful and toxic marine algae has recently gained a growing public and scientific interest both in Ireland and abroad because of the occurrence of these toxins in shellfish.

What are toxic phytoplankton?

Phytoplankton are microscopic single-celled algae. In temperate waters the phytoplankton can be divided into two groups, diatoms and flagellates, depending on the their ability to swim.

The swimming phytoplankton are called flagellates. These swim by means of one or more whiplike extensions ("flagella") that beat very rapidly to propel the cell through the water. Diatoms are non-swimming phytoplankton that depend upon water movements for their transport.

Dinoflagellates (with two of these flagellae) are perhaps the most important flagellates from a public health and shellfish producers point of view because of their involvment in toxin production. Marine bio-toxins produced by dinoflagellates may be responsible for toxic effects on humans and other animals.

Bivalve shellfish (e.g. mussels, oysters) feed directly on phytoplankton, using their gills as sieves to strain the cells out of the water. Because bivalve shellfish filter many litres of water daily they can concentrate phytoplankton cells which may be present at low levels. Thus, even if toxin producing species are present in low numbers, the shellfish can still accumulate the toxins rapidly.

The toxins normally do not affect the shellfish themselves but upon consumption by upper trophic levels including humans, can result in various forms of shellfish poisoning including diarrhetic shellfish poisoning (DSP), paralytic shellfish poisoning (PSP), amnesic shellfish poisoning (ASP), and neurotoxic shellfish poisoning (NSP). Continual monitoring of both shellfish and seawater ensures the quality and safety of Irish shellfish being placed on both the domestic and export markets.

Which species are toxic?

PSP: Paralytic Shellfish Poisoning is a global phenomenon and PSP type illnesses have been documented since the1700s. Produced by phytoplankton dinoflagellates of the *Alexandrium, Pyrodinium* and *Gymnodinium* genera, the paralytic shellfish poisons are also found in large concentrations in bivalve shellfish that feed on the microalgae. One of the genera implicated in P.S.P. are the *Alexandrium* species. These have been shown to produce saxitoxin, a known P.S.P. neurotoxin. Although a frequent member of the summer phytoplankton assemblage, they do not appear to be particularly toxic in Irish shellfish. PSP has been described in Belfast Lough and also in Kerry in the 1800's. One borderline incidence was detected in Cork Harbour in 1987 during a bloom of *Alexandrium* and this necessitated a closure of the area. However *Alexandrium* (PSP) and *Dinophysis* (DSP) do not have to be in bloom concentrations to produce shellfish toxicity so all incidences of these species are tested to see if shellfish toxicity is present.

ASP and NSP: *Nitzschia pungens* var. *multiseries* is responsible for Amnesic Shellfish Poisoning. This is the only recorded diatom producing a neurotoxic amino acid namely Domoic acid. An outbreak in autumn 1987 resulted in 107 cases of ASP in Prince Edward Island, Canada. Three deaths were directly associated with the outbreak. Fortunately, this form of shellfish toxicity has not been detected in Ireland.

Ptychodiscus brevis which produces N.S.P. (mainly in south eastern USA) is also absent from Irish waters. Impacts of this organism include massive coastal fish kills and shellfish poisonings. This is the only known organism to produce a toxic aerosol that is irritating to human mucous membranes.

DSP: Japanese studies in the 1970`s indicated a close correlation between the dinoflagellate *Dinophysis fortii* and shellfish induced gastrointestinal tract symptoms. Consequently the toxin was named Dinophysistoxin and the syndrome was given the name diarrhetic shellfish poisoning (DSP)

The picture has been further complicated by the discovery of several toxins in the DSP complex. These are subdivided into three groups; (a);Okadaic acid (OA) and the closely related dinophysistoxins (DTX), (b);Pectinotoxins (PTX 1-3) and (c); the yessotoxins (YTX) .

The most commonly occurring DSP phycotoxins in Irish shellfish are okadaic acid and dinophysistoxin-2. These originate from *Dinophysis acuminata* and *Dinophysis acuta,* which are dinoflagellates. Both occur widely during the Spring and Summer on the western and southern coast and have been found in shellfish guts in high numbers during toxic episodes. Although they are normally found at low levels (<400 cells / l) throughout much of the summer occasional

summer blooms of the species may occur (>30,000cells / l). For the past 10 years there has been an annual closure of Irish shellfisheries due to *Dinophysis* induced D.S.P., except for 1985 and 1986. Another D.S.P. producer, the benthic/epiphytic species *Prorocentrum lima* also occurs but so far has not been implicated in the toxicity of Irish shellfish.

Although the DSP toxins are extracted by the same procedure (with acetone) only OA and DTX are associated with diarrhoea. The PTX and YTX compounds although acutly toxic to mice upon i.p. injection, (as are OA and DTX) they do not induce diarrhoea. Consequently it is only logical to include just OA and DTX in the true DSP toxin complex. A new marine toxin DTX-2 was isolated and identified from toxic Irish mussels during the course of our routine monitoring for DSPs in mussels in Bantry Bay. This was the first recorded occurrence of the toxin and completed the DTX suite of compounds. It has subsequently identified in shellfish in Spain and Portugal, and probably occurs elsewhere

Toxicity: The physiological effects of okadaic acid have been described by many researchers. The genaral effects of i.p. injection of 200 $\mu g\ kg^{-1}$ O.A. into mice are general weakness and inactivation. The effects appear 30 mins to several hours after injection, and at sufficiently high concentrations the animals die between 1hr 40 mins and 47 hrs. When the algal toxins are given by the oral route the lethal dose is approx 16 times higher but the symptoms are the same. Similar effects are obtained from DTX-1

The reason for this is that the toxin degenerates the absorptive epithelium in the intestine. Following injection of OA into the the middle duodenum of rats, changes were apparent after 15 minutes. The enterocytes at the tips of the villi become swollen and subsequently detached from the basal membrane. I.V. injection induced similar but less extensive changes indicating that the enterocytes are specific targets for OA.

The mechanism for this pathological change has been shown to be stimulation of phosphorylation of proteins that control sodium secretion by intestinal cells. Phosphorylation and dephosphorylation are among the most important regulatory processes in eucaryotic cells controlling processes as diverse as metabolism, membrane transport and sectetion, contractility and even cell division. The extent of the damage in rat small intestine is dose dependant, 3ug will affect the tips of the villi, while 5ug can lead to the collapse of the villous structure

Symptoms in Humans:The first known incidence of DSP in humans was reported in the 1960`s in the Netherlands. Similar symptoms were reported in the 1970`s from Japan. The dominating symptoms in Humans were diarrhea, nausea,

vomiting and abdominal pain. Within a few days the victim recovered with no after effects.

The intensity of the symptoms in humans depends on the amount of toxin ingested. Usually even though the patients feel very sick, hospitalisation is not necessary.

Extent of the problem:

Subsequent to the initial reports in the `60s of DSP in the Netherlands and Japan, several similar reports were obtained from different parts of the world. DSP is now a worldwide phenomenon. It has been detected in Ireland since 1984.

The DSP incidences or at least the detection of DSP toxins in shellfish appear to be increasing. It should be noted that toxin producing algae and toxic molluscs are frequently reported from new areas, and this spreading may be one of the reasons for increased incidence of human intoxications. This may be partly due to increasing knowledge about DSP and/or better surveillance programs.

In Ireland the length of time an area is closed varies and the decision to re-open is taken only if samples give negative bioassay results for two consecutive weeks. Over the last 10 years the duration of the closure of shellfisheries has varied from year-to-year.

Duration of closure of shellfisheries due to DSP, 1984-1993

YEAR	DURATION OF CLOSURE
1984	August - October
1985	No closure
1986	No closure
1987	August- December
1988	June - November
1989	June - September
1990	July - October
1991	June - November
1992	June - September
1993	June - September
1994	May - December

Monitoring:
Unfortunately, seafood contaminated with algal toxins may appear wholesome even though its` consumption may cause illness. Effective monitoring of these products ensure that they are not placed on the market.

The Fisheries Research Centre of the Department of the Marine is an EU designated National reference Laboratory on Marine Biotoxins, (Council Decision 93/383 EEC) The laboratory operates a national monitoring program under EC directive 91/492 and EC directive 91/493.involving the testing of shellfish for the presence of toxins and the analysis of water for the presence of toxin producing algae. Experience ove the past ten years has shown that the phytoplankton that produce ASP and NSP do not occur frequently in the plankton. Blooms of *Alexandrium* which can potentially produce PSP toxins have been recorded on two occasions, Cork Harbour in 1987 and Lettercallow in 1992. PSP toxins have only been detected on one occasion at very low levels in Cork Harbour in 1987. Subsequent PSP tests from shellfish growin areas all over Ireland have yielded negative results.

The dinoflagellates *Dinophysis acuminata* and *Dinophysis acuta,* which are associated with outbreaks of DSP, do however, occur regularly in the plankton during the summer. These results indicate that the main potential problem with bivalve shellfish from Irish coastal waters, from a public health point of view, is with DSP. The monitoring programme and the methods used have thus focused on the detection of DSP toxins in shellfish.

Methods:

a; Phytoplankton; the number of cells necessary to induce toxic mussels varies considerably. The species of *Dinophysis* also appears to affect both the type of toxin present and also the levels of toxin. The proportion of non-toxic species present in the water alongside *Dinophysis* also has a marked effect on the intoxification rate of the shellfish.

A reliable phytoplankton sampling regime is necessary to establish thresholds for early warning to shellfish growers. This gives important information regarding the rates of intoxification and detoxification.

Samples of seawater from several depths are monitored weekly in FRC for the presence of toxic and other nuisance species. A database of cell-counts has been established since 1989 yielding valuable trend information.

b; Shellfish; Tests to detect and measure phycotoxins in shellfish vary in sensitivity, speed, cost and ability to detect more then one type of toxin. The tests used at present fall into two basic categories: tests using animals (bioassays) and chemical analysis.

Biological Methods;

At the Fisheries Research Centre an ingestion-diarrhea rat bioassay is used. As the toxins are concentrated in the hepatopancreas of the shellfish this organ is used in the bioassay. Ten grammes of mussel hepatopancreas are offered to pre-starved (24 hr) Wistar laboratory rats. The following day the consistency of the faeces is examined and scored from 0 (normal) to 4 (diarrhetic). If the faeces are other than normal then this suggests that DSP toxins are present.

Chemical Methods

Chemical analysis of the toxins also involves removal of the hepatopancreas from the mussel sample. The toxins are then extracted into an organic solvent and, following cleanup to remove unwanted co-extracted material, the extract is analysed using Liquid Chromatography-Mass Spectrometry (LC-MS) Identification and quantification of the toxins present is achieved by comparison with pure standards of the toxin. The LC-MS method was introduced in 1994 and has been found to be superior to the previously used HPLC method.

Restrictions placed upon harvesting:

EC Directive 91/492, which lays down the health conditions for the production and the placing on the market of live bivalve molluscs, states that " *the customary biological testing methods must not give a positive result to the presence of Diarrhetic Shellfish Poison (DSP) in the edible parts of the molluscs (the whole body or any part edible separately).*" Thus, in order to comply with this directive and to ensure that shellfish containing toxins are not placed on the market, restrictions on the harvesting and sale of shellfish are put in place when bioassays give positive results. Each of the relevant Health Boards are informed of the results of the bioassays. If results are positive then the Health Boards issue public notices restricting the harvesting and sale of shellfish from specific areas. As yet there is no satisfactory method to detoxify the shellfish in order to reduce the duration of closures. The only successful method is to allow the shellfish to detoxify naturally. Rates of detoxification appear to depend on a

number of factors including the numbers of non-toxic phytoplankton species available and the temperature of the water.

From a shellfish producers point of view, protracted closure of an area can cause economic hardship due to loss of markets as continuity of supply can not be guaranteed and loss of product can occur due to slippage from ropes. However, a shellfish poisoning incident can effect consumer confidence in the industry for many years as a result of media attention, sensationalism and often misinformation. The importance of protecting the consumer from phycotoxins in shellfish cannot be underestimated. It must also be emphasised to the consumer, however, that the risk of contracting shellfish poisoning are very small due to the efficacy of the monitoring and testing procedures in place in Ireland.