IRISH SHELLFISH BIOTOXIN MONITORING PROGRAMME

Joe Silke, Terry Mc Mahon and Philipp Hess
Marine Institute, Galway, Ireland.

Introduction

Since its initial development in the early 1970s the Irish aquaculture industry has grown to be an important contributor to the national economy. There has been a steady increase, in both output and value, as well as in job creation. The total production of farmed shellfish has increased from approximately 5,000 tonnes in 1980 to 44,678 tonnes in 2003 (Figure 1), with a first sale value of €41.8m and directly employing some 1100 people (Parsons *et al*, 2004). Mussels (*Mytilus edulis*), native oysters (*Ostrea edulis*), Pacific oysters (*Crassostrea gigas*), Clams (*Tapes semidecussata*) and scallops (*Pecten maximus*) are the main species produced. With a growing recognition and awareness internationally of the potential human health effects of the consumption of shellfish containing algal toxins, a monitoring programme was established in Ireland in the early 1980s and has continued since then. In this paper the evolution and development of the programme is described and discussed.

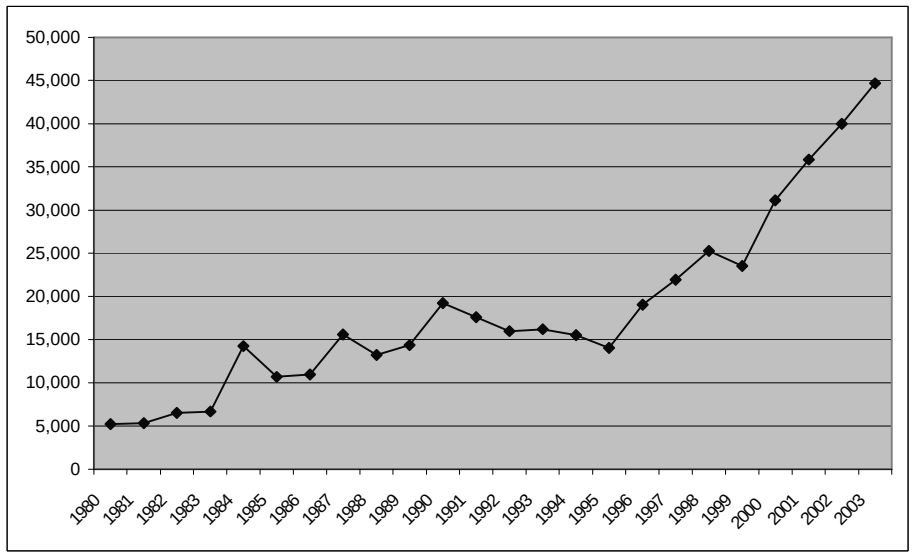

Figure 1. Shellfish production(tonnes) in Ireland 1980 – 2003

Lipophilic toxins

In the period up to the early 1980s, shellfish toxicity leading to diarrhetic shellfish poisoning (DSP) occurred so rarely in Ireland that it failed to provoke any action on the part of the regulatory authorities. However, following the first publication in the scientific literature showing the association between the occurrence of *Dinophysis spp*.and the accumulation of DSP toxins in mussels in The Netherlands (Kat, 1983) and the first records of *Dinophysis* in Ireland made at the Sherkin Marine Station on the southwest coast, the Irish Biotoxin and Phytoplankton Monitoring programme was established. The initial testing programme consisted of observations of phytoplankton samples on field trips made by Department of Fisheries staff to the southwest and the use of Rat bioassay for testing the shellfish. This testing

continued through the late '80s in the southwest, mainly in the summer months. With the expansion of the industry in the early part of the 1990s along the south and west coasts, the volume of sampling and analysis also increased. In 1991 European legislation (Council Directive 91/492/EEC) was introduced to govern the placing on the market of live bivalve molluscs and to control the toxicity therein. This Directive was subsequently amended by Commission Decision 2002/225/EC to take account of improved analytical methodologies and increased knowledge on toxicology. Initially in Ireland Okadaic Acid was shown to be the main DSP toxin present but in 1991 Dinophysistoxin-2 (DTX2) was identified for the first time in mussels from Bantry Bay on the southwest coast (Hu *et al*, 1992, Nixon and Taffe, 1993). Subsequently the toxins were monitored by bioassay and quantified by HPLC methods. In 1994 positive bioassays, due to the presence of DTX2 at high levels (McMahon *et .al,* 1996), persisted through the winter months (Figure 2) resulting in a protracted ban on the harvesting and sale of shellfish from these areas with significant financial losses for the industry. Following these protracted closures a Ministerial Task Force was established to review the monitoring programme. Recommendations of the review included year round testing of samples and the replacement of the rat bioassay with the Yasumoto 1978 mouse bioassay which was being used in most EU Member States at that time. In 1996 The Marine Institute also took over the role of coordinating the testing from the Department. By the late 90's up to 4000 bioassays were being carried out *per annum*, and a phytoplankton monitoring programme was also in place around the coast

In November 1995 at least 8 people became ill in The Netherlands following the consumption of mussels harvested from Killary Harbour, on the west coast of Ireland and subsequently a previously unknown toxin, Azaspiracid, was isolated and identified (Satake *et al*, 1998) This event marked a watershed in the monitoring programme and the reliance on bioassay testing alone was questioned. European Union legislation specified that a bioassay is to be used as the reference method, but alternative methods that could provide an equivalent level of human health protection, could be incorporated into the monitoring programme. The Irish programme changed to a 24 hour Mouse Bioassay including a clean-up step, in line with the method most widely used in Europe, and liquid chromatography mass spectrometry (LC-MS) was introduced in 2001 to allow for the identification and quantification of toxins present and chemical confirmation of the bioassay results, initially to reduce and eventually to move towards replacing animal based testing.

Table 1 shows the percentage positive bioassays observed for all species since 1994. The earlier years have high percentage positive results, but this is due to the emphasis of testing the southwest mussels alone and only in the summer months. The overall picture here however does show that there are some years when there is very little toxicity present, as low as 1.5 % for all species, but other years can be much higher, and especially in the summer months. No obvious pattern is present for these variations such as correlation to high rainfall years, warmer summers etc. A similar pattern is evident, albeit at a lower level with PSP toxicity. These figures are based on all shellfish species tested, and as rope-grown mussels are more prone to toxification than the other commonly farmed species such as oysters and clams, there may therefore be a much higher incidence of toxicity in these shellfish.

Table 1. Percentage of Positive Bioassays 1994 -2002

Year	Total DSP Bioassay	% Positive	Total PSP Bioassay	% Positive
1994	778	61.3		
1995	611	29.3		
1996	343	13.1		
1997	755	1.7	140	1.4
1998	1010	1.5	93	1.1
1999	1488	6.9	17	0
2000	2991	18.1	27	7
2001	4030	16.3	217	0
2002	2494	3.4	124	3.2

The question remains, why are these inter annual differences present, and in the absence of geographical, or inter-annual patterns the primary suspect is variability in the presence and dominance of toxic phytoplankton. The most important of these is probably the subtle switch from diatom-dominated phytoplankton in the early summer to a dinoflagellate-dominated community. The time of this switch and the ratio is different each year. Observing this however demands a high frequency phytoplankton-monitoring programme with many more sampling points and some offshore sampling. The impact on production is dramatic, and the variability from year to year is shown for the Bantry area in Figure 2. One of the difficulties with shellfish toxins is the unpredictability of the time of onset, the intensity and the duration of the event. It ranges from years that required no closures to a continuous closure of 10 months between 1994 and 95. This makes it very difficult for producers to guarantee delivery to their customers especially at shoulder periods of toxicity. Obviously, an industry that is operating under such a variable window of opportunity requires much assistance in forecasting the onset and duration of these toxic events.

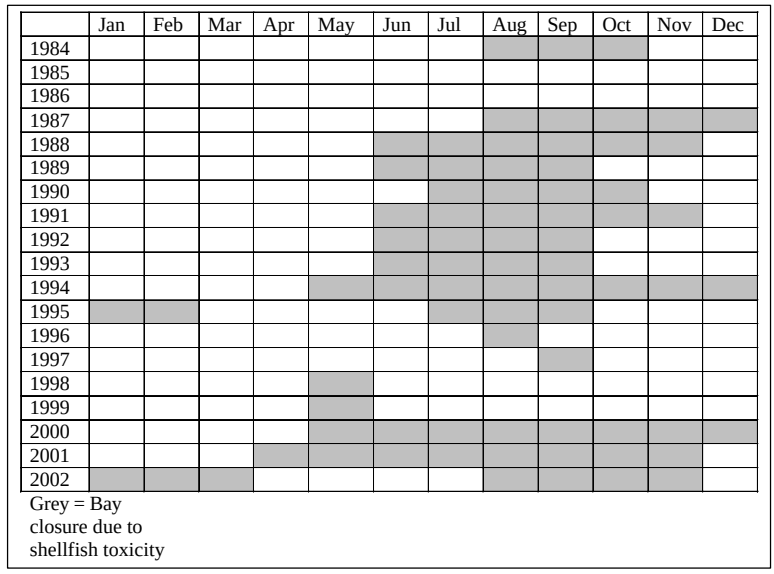

Figure 2. Variability in annual closures in Bantry Bay, SW Ireland, 1984 -2002

Current DSP Monitoring Programme

Following the operational changes outlined above, regionally based laboratories were contracted to carry out routine bioassay testing with defined quality control procedures and a laboratory accreditation plan was initiated. Approximately 2200 samples per annum are now analysed by bioassay in the regional laboratories, with chemical (LC_MS) backup being carried out in the Marine Institute. Rapid turnaround of the results of this testing programme was facilitated by the incorporation of modern communications technology to speed up the transfer of data and information between laboratories, regulatory authorities and the industry (Figure 3). This was implemented by the development of an online database, which can be accessed by the regional laboratories through the Internet. The database facilitates the decision making process as it can present all of the necessary information both current and historical in a suitable report format to help decide the status of a given shellfish production area. These reports are then issued to the various interested parties via web, fax, SMS and email. The objective is to report the result of the analysis of all samples submitted within 3 days of the samples being received in the laboratory. The development of the database system has greatly facilitated this objective, with over 95 % of test results meeting the 3-day turnaround deadline.

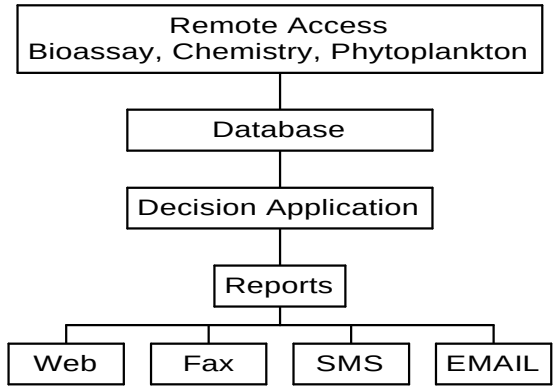

Figure 3. Biotoxin Information System

Amnesic Shellfish Toxins:

The content of domoic acid (DA) in shellfish intended for human consumption has also been regulated in the EU by the Council Directive 91/492/EEC, as amended by Council Directive 97/61/EC and Commission Decision 2002/226/EC. The maximum concentration permissible for DA in marketed shellfish or shellfish products is set to 20 mg/kg of the whole flesh or any edible part separately. The Marine Institute is responsible for the routine surveillance for amnesic shellfish poisons (DA and isomers) in live bivalve molluscs in Ireland. This monitoring entails testing of ca. 700-800 tissue samples per annum, mostly arising from the control of scallops (*Pecten maximus*) landed as fisheries products, although other shellfish species are also tested at reduced frequency. The highest concentrations of DA in shellfish from Irish waters are typically found in scallops, particular in the hepatopancreas, leading to situations where scallops must be processed to achieve toxin-free edible parts such as adductor muscles and gonads. Due to the food safety aspects and the requirement for freshness, quality of results and a fast sample turnaround are important factors in the official control of DA.

The ASP testing protocol used in Ireland, based on the EU Directive, is a three-tiered status for the individual fisheries.

Open	DA in each compartment of the scallop i.e.Adductor Muscle, Gonad, Hepatopancreas and Total Tissue is less than 20mg/Kg
Closed	DA in the adductor Muscle and/or gonad is greater than 20 mg/Kg
Limited Sale	Where DA in the Hepatopancreas is greater than 20 mg/Kg but DA in both Adductor Muscle and Gonad is less than 20 mg/Kg sale to approved processing establishments may be allowed for shucking Adductor Muscle and Gonad must be re-tested before placing on market

This scheme allows some fishing to continue in the presence of DA in the non-edible parts of the scallop. In practice, as the levels of DA in these parts of the scallop has typically been above the regulatory limit since monitoring began in 1999, the bulk of Irish fished scallops are shucked and the edible parts only are submitted for testing. These tests on representative samples from the processed batch ensure that only shucked meats below the safety threshold of 20ug/g are placed on the market.

Paralytic Shellfish Poisoning

The third group of toxins occasionally present in Irish shellfish is the Paralytic Shellfish Poison group (PSP). These are monitored in response to the presence of *Alexandrium* in seawater, using the standard AOAC PSP bioassay. In addition, the use of Immunoassay kits are being explored as valid screening alternatives to this test. The threshold of 80ug STX eq./100g flesh is used as the closure level and this has only been observed regularly as a summer phenomenon in the area of Cork Harbour on the South coast. In this area, mussels exhibit levels requiring action for a short period most years, and occasionally flat oysters are also affected. The presence of PSP in shellfish outside of Cork Harbour has been observed rarely.

Risk Based Management

In the area of food safety, the public often supports strong policies, hoping to reduce or eliminate risks to human health. But recent evidence indicates that some of these policies are not directed at the most significant sources of risk. The effect of such policies may be to misallocate resources that could improve public health if those resources were directed toward larger risks. During the early 1990s the allocation of resources to shellfish safety was totally inadequate. With the detection of Azaspiracid in the mid 90's and the extended closures in 2000-2001, pressure was placed by the industry on government to re-allocate resources and promote more informed decision-making about shellfish safety through greater use of tools such as risk assessment and decision analysis. Under the guidance of a committee of Regulators and Industry representatives (The Molluscan Shellfish Safety Committee or MSSC) a re-vamped programme was implemented in 2001 to provide sensible consumer safety but also to protect the industry from unnecessary closures. A management concept taking a holistic view of risk was initiated in 2003. Shellfish production closure and opening would now be based on all information available from bioassay, chemistry, phytoplankton, recent history and results from adjacent areas. A Management Cell, made up of representatives from the Food Safety Authority, Department of Communications, Marine and Natural Resources, Irish Shellfish Association and the Marine Institute, was established to discuss compiled information, and in the case of unusual or un-seasonal results take a measured decision. A range of improvements to the process came with the adoption of risk based management techniques. While the EU directives ultimately governed the

amount of flexibility that could be introduced into the system, a more pragmatic and appropriate level of control was introduced with the combined interest of consumer food safety and industry interests taken into account.

Conclusion

The establishment of a suitable monitoring programme is dependant on the implementation of many diverse elements. The Irish National Biotoxin Monitoring Programme is an effective implementation of phytoplankton, bioassay and biotoxin chemistry monitoring programmes. Each of these has their own strengths and weaknesses but in combination they provide a very strong programme to protect human health. Phytoplankton monitoring may provide an early warning of potential biotoxin contamination, often with results available before the shellfish tests. Identification of toxic species alone can trigger an action plan to delay harvest, or in some cases to close an area and thereby protect human health. However the patchiness of phytoplankton in the water makes it very difficult to obtain representative samples. Bioassays can provide a good indication of overall toxin load in shellfish, and some indication as to the safety of the shellfish when the toxicology of the toxin is unknown. In certain cases the bioassay can be calibrated to give a semi quantitative approximation of toxin equivalents in the shellfish and the test does not require sophisticated equipment. There is, however, some evidence that bioassays may be oversensitive to certain toxins and the reliance on bioassays alone for regulatory decision-making can be questioned. Chemical analytical techniques offer extremely sensitive methods for the quantification of the presence of biotoxins in shellfish. In many cases these methods are the only means of determining the identification of the particular toxin(s) present. In addition, due to their sensitivity these methods can detect toxin levels well below the threshold of closure, thus sometimes offering forecast information on the onset of the problem. The success of any biotoxin monitoring can be judged in terms of how consumer safety has been ensured. Since the restructuring of the programme in Ireland in 2000/2001 there have been no reports of human illnesses or product recalls associated with biotoxins in Irish Molluscan shellfish on national or international markets. The programme will continue to improve and adopt state of the art methodologies and management concepts into the future.

References

Hu, T. Doyle, J., Jackson, D., Marr, J., Nixon, E., Pleasence, S., Quilliam, M.A., Water, J.A. and Wright, J.L.C. 1992 Isolation of a new diarrhetic shellfish poison from Irish mussels. Journal of the Chemical Society Communications **30**: 39 –41

Kat M., (1983) Diarrhetic mussel poisoning in the Netherlands related to the dinoflagellate *Dinophysis acuminata*. Antonie Van Leeuwenhoek **49**, 417

McMahon T., Silke J., Nixon E., Taafe B., Nolan A., McGovern E., and Doyle J. (1996): Seasonal variation in Diarrhetic Shellfish Toxins in mussels from the southwest coast of Ireland in 1994.In: Keegan B. (Ed.) Proceedings of the Irish Marine Science Symposium, Galway 1995. UCG Press, Ireland.

Nixon. E. and Taffe, B 1993. DSP toxins in mussels and the contribution of a new toxin, DTX2. International Council for the Exploration of the Sea CM 1993/F:28

Parsons A., O'Carroll T., O'Cinnéide M. and Norman M. (2004) Status of Irish Aquaculture 2003, Marine Institute Publications, Galway Ireland.

Satake, M., Ofuji, K., Naoki, H., James, K.J., Furey, A., McMahon, T., Silke, J and Yasumoto, T. 1998 Azaspiracid, a new marine toxin having unique spiro ring assemblies, isolated from Irish mussels, Mytilus edulis J. American Chemical Society **120**: 9967-9968