A Review of the Second International Conference on Molluscan Shellfish Safety

Seafish Report No. SR512

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The Sea Fish Industry Authority

Seafish Technology

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Summary

The Second International Conference on Molluscan Shellfish Safety was held in Iloilo City, The Philippines on 17 - 21 November 1997. This was the fourth in a series of conferences relating to Molluscan Shellfish Safety, the others being The First International Conference on Molluscan Shellfish Safety in New South Wales, Australia in 1994 and two further conferences in 1998 and 1992 on Shellfish Depuration in the United States of America and France.

The scope of the conference encouraged a range of food safety related issues to be raised and provided researchers and industry with a picture of the range of factors which can affect shellfish safety. This report contains the abstracts of the papers and in some cases a precis of the presentations. The conference recommendations are also reported. An appendix outlining some of the illness associated organisms detailed in the papers is also included.

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Appendix No. I A Summary of the Illness Associated Organisms which can Affect Molluscan Shellfish Safety

1. Introduction

The Second International Conference on Molluscan Shellfish Safety was held on 17-21 November, 1997. This was the fourth in a series of conferences relating to Molluscan Shellfish Safety, the others being the First International Conference on Molluscan Shellfish Safety in New South Wales, Australia in 1994, and two further conferences in 1988 and 1992 on Shellfish Depuration in the United States of America and France. The scope of the conference encouraged a range of food safety related issues to be raised and provided researchers and industry with a picture of the range of factors which can affect shellfish safety.

This conference was held in Iloilo City, The Philippines with approximately 77 delegates from 15 countries. It was organised by The University of the Philippines with an International Advisory Committee from Australia, New Zealand, Japan, The Philippines, United States of America and France.

The abstracts of the papers and in some cases precis are reported. Whilst every effort has been made to ensure the precis are as accurate as possible some discrepancies might occur. The full papers should be published by the conference organisers by the middle of 1998. These are to be published in the Journal of Shellfish Research. The editor Sandra E. Shumway attended the conference.

An Appendix outlining the illness associated organisms associated with bivalve molluscs is also included.

2. Papers on Bacterial and/or Viral Related Issues

2.1 The Scottish Approach to the Application of Microbiology Aspects Relating to Shellfish Directive 91/942/EEC

SUSAN GALLACHER*, J. GRAHAM and F.G. HOWARD

FRS Marine Laboratory, P.O. Box 101, Victoria Road, Aberdeen, Scotland, UK

ABSTRACT:
EC Shellfish Directive 91/492/EEC, lays down the health conditions for the production and placing on the market of live molluscs. Under this directive and associated UK legislation, the competent authority determines the boundaries of production areas, and classifies these sites according to the degree of contamination using the faecal indicator *E. coli*. This presentation will describe the establishment of the programme in Scotland, the classifications issued, factors which can influence results and research into improving the methodology used.

KEYWORDS: EC Shellfish Directive 91/492/EEC, *E. coli*, faecal indicators

UK SUMMARY:
There are 175 shellfish growing sites in Scotland. 7,000 microbiological samples have been assessed over the last 5 years from these sites. The assessments show that 55% of sites are category 'A', 20% A/B, 20% B and 5% C.

There can be problems with microbiological assessments. For example on mussel long lines, mussels from the top of the line can be category 'B' and from the bottom of the line category 'A'. There are also problems with the *E.coli* MPN test itself. A multiple test trial using a category 'A' homogenate of shellfish found that one third of the analyses gave a category 'B' result. There is a need for a better *E.coli* test such as the new Chromocult (Merck) or Neogen tests.

*The name in bold indicates the presenter of the paper

2.2 Human Enteric Viruses in Oysters Causing a Large Outbreak of Human Food Borne Infection in 1996/97

BIRGITTE F. CHRISTENSEN [1], D. LEES [2], K. HENSHILWOOD [2], T. BJERGSKOV [1] and J. GREEN[3]

[1] *Danish Veterinary and Food Administration, Ministry of Food, Agriculture and Fisheries, Rolighedsvej 25 1958 Frederiksberg C, Denmark*

[2] *Centre for Environment, Fisheries and Aquaculture Science, Barrack Road, The Nothe, Weymouth, Dorset DT4 9TH, UK*

[3] *Virus Reference Division, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT, UK*

ABSTRACT:
During New Year 1996/97, more than 350 persons in Denmark were ill due to consumption of imported oysters. The main symptoms were vomiting, diarrhea, abdominal pain and fever, commencing 12-48 hours after consumption. In addition, a number of the diseased persons reported of secondary symptoms, such as aching of joints, numbness of skin and visual disturbances, commencing 24 hours after onset of the primary symptoms. In general, the recovery from both the primary and in particular the secondary symptoms was slow. Only 3/24 (12%) of the analysed samples of infected oysters showed an *E. coli* level above the allowed regulatory limit. A small amount of domoic acid was found in 4/9(44%) of analysed samples. Small Round Structured Virus (SRSV) and enterovirus were identified from both oyster and faecal samples using RT-PCR. Enterovirus isolated from individual faecal samples showed sequence identity of 3 PCR amplicons, suggesting a common source of infection. The identity of the enterovirus is still under investigation. The characteristic clinical symptoms and RT-PCR results implicate SRSV's as the cause of the primary symptoms. However, the cause of the secondary symptoms is currently unclear. Although the detection of enterovirus in both faecal samples and oysters is significant, the incubation period prior to onset of secondary symptom was not typical of an enterovirus infection. The significance of the domoic acid in relation to the outbreak is unclear.

KEYWORDS: enteric viruses, human food borne outbreak, oysters

UK SUMMARY:
An EU investigation found documentation fraud, mixing of shellfish batches and had concern about the presence of domoic acid (Amnesic Shellfish Poisoning, ASP). This has led to a new EU directive 97/61/EC, which aims to tighten up on these issues when it comes into force on 1st July 1998. The investigation also highlighted the problems of *E.coli* as an indicator of viral contamination. The EU have set up a chain of nominated national virus reference laboratories and CEFAS, Weymouth has been nominated the EU central reference laboratory.

2.3 Relationship between Winter Epidemic of Acute Gastroenteritis in French Population and Shellfish Viral Contamination

LAURENCE MIOSSEC, FRANCOISE LE GUYADER, LARISSA HAUGARREAU, MARIE-ANNICK COMPS and MONIQUE POMMEPUY

Laboratoire de Microbiologie, Nantes et laboratoire, Cotier de Sete, BP 21 105, Cedex 3, IFREMER, Nantes, France

ABSTRACT:
Several gastroenteritis outbreaks, linked to shellfish consumption, have been reported worldwide in the literature. Different shellfish species were involved, but more frequently those eaten raw. In Europe, few gastroenteritis outbreaks, following shellfish consumption and suspected to be human calicivirus infections, have been observed for a few years always during winter. However few data are available on viral contamination occurrence in shellfish. First results of a field survey are presented on viral contamination in two shellfish harvesting areas extending along the French Mediterranean coast. The first one, mainly oyster beds, is classified in category 'A', as determined by faecal conform counts in shellfish (EC Directive 91/492). The second one is a mussel bed classified in category 'B'. Shellfish samples were collected monthly between August 95 and April 97. RT-PCR was used to detect viruses known to be involved in gastroenteritis outbreaks: enterovirus, human calicivirus, rotavirus and astrovirus. Faecal contamination expressed as faecal conforms was evaluated in the same samples. Virological results in shellfish were correlated with incidence data of gastroenteritis epidemic among coastal population, obtained from the French Sentinelle System for Surveillance of Communicable Diseases. A relationship was observed between virological results and epidemiological data. For the two years, when gastroenteritis incidence rate was maximum in winter, the mussel bed was always contaminated by the four types of viruses searched. For oyster beds, the same results were observed the second winter, however during the first winter, two samples were highly contaminated whereas the third one presented low contamination (only rotavirus). These results allowed to hypothesize that diarrhea epidemic in the French population would contribute to the viral contamination of the marine environment through waste water dispersion.

KEYWORDS: shellfish, viral contamination, enterovirus, human calicivirus, rotavirus, astrovirus, faecal coliform, gastroenteritis epidemic

UK SUMMARY:
A survey was done on 3 category 'A' gigas sites and 1 category 'B' mussel site. Viruses were found in all sites during the autumn and winter. A relationship was shown between virus levels in the shellfish and epidemiological evidence of gastroenteritis in the population. This reached levels of 200 people per 100,000.

There is a need for a better viral classification indicator and more research into viral depuration of bivalves.

2.4 Application of a Nested Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) Assay for the Detection of Small Round Structured Viruses (SRSVS) in Environmentally Contaminated Molluscan Shellfish

KATHLEEN HENSHILWOOD [1], J. GREEN [2], C. L. GALLIMORE[2], D. W. G BROWN [2] and D.N. LEES [1]

[1] *Centre for Environment, Fisheries & Aquaculture Science, Weymouth Laboratory, Barrack Road, The Nothe, Weymouth, Dorset, DT4 8UB, UK*

[2] *Enteric and Respiratory Virus Laboratory, Virus Reference Division, Central Public Health Laboratory, Colindale, London, NW9 5HT*

We describe the evaluation of a nested RT-PCR procedure for the detection of SRSV shellfish and the application of this assay for the detection of SRSV in commercially produced shellfish and in shellfish implicated in outbreaks of gastroenteritis. The range of strains detected and the sensitivity of detection were evaluated using a panel of 21 well-characterized SRSV strains. The nested RT-PCR detected 15 of the SRSVs demonstrating that the assay detects a broad range of SRSVS including strains from genogroups I and II. Seeding experiments showed the nested RT-PCR assay to be 10-10^4 times more sensitive than the single-round RT-PCR assay for the detection of SRSV in shellfish. When used to test shellfish samples from polluted harvesting areas and shellfish samples implicated in outbreaks of gastroenteritis, SRSV-contaminated samples were identified by nested RT-PCR which were which were negative by single round RT-PCR. The assay was used to investigate the effect of relaying contaminated shellfish in clean water ponds and demonstrated that a minimum period of four weeks followed by purification yielded SRSV-negative oysters as judged by nested RT-PCR. This assay has potential applications for monitoring "at-risk" shellfish harvesting areas, for investigation of SRSV contamination in shellfish from producers linked to gastroenteritis outbreaks and for the direct detection in shellfish implicated in outbreaks.

KEYWORDS: virus, shellfish, nested PCR, SRSV

2.5 Better Assessment of Viral Hazard from Molluscan Shellfish Consumption

DAVID N. LEES, W. J. DORE and K. HENSHILWOOD

Centre for Environment, Fisheries and Aquaculture Science, Weymouth Laboratory, Barrack Road, The Nothe, Weymouth, Dorset, DT4 8UB, UK

ABSTRACT:
EU public health regulations for molluscan shellfish specify that shellfish sold for consumption must meet a bacteriological standard of <230 *E. coli*/100g of shellfish and absence of Salmonella in 25g. In the UK, and elsewhere in Europe, purification of shellfish in tanks of clean seawater is used extensively to meet this standard. However it is well documented that oysters purified in this way, and meeting the bacteriological standard, may still cause viral gastroenteritis if consumed raw. The limitations of traditional bacterial standards are well known and have prompted the evaluation of alternative measures for evaluating viral risk associated with shellfish. We report on the development of an alternative "viral indicator" capable of more accurate assessment of viral contamination of shellfish and on the validation of this indicator using PCR methods for Small Round Structured Viruses (SRSV's) responsible for consumer illness. The results showed whilst levels of *E. coli* in shellfish sold for consumption were routinely compliant with legislative standards, male-specific bacteriophage levels varied from <30 to over 10,000 pfu/100g. High phage levels were shown to be consistent with SRSV contamination in a small number of samples. Results suggest that the male-specific bacteriophage, but not the *E. coli,* content of shellfish is a reliable guide to the likely food hazard and that absence of male-specific bacteriophage in 100 g of shellfish flesh may provide an adequate indication of food safety for human viruses.

KEYWORDS: shellfish, male specific bacteriophage, SRSV

UK SUMMARY:
F+ bacteriophage has been shown to be the most representative indicator of SRSV's in shellfish and growing waters in England and Wales. Depuration studies show differences in *E.coli* and F+ phage removal from shellfish. The time for 90% reduction of the organism was found to be 4.5 hours with *E.coli* in mussels but 47.3 hours for F+ phage and 6.5 hours with *E.coli* in gigas but 54.6 hours for F+ phage. Analysis of retailed depurated gigas samples from category 'B' areas has shown low *E.coli* levels but higher levels of F+ phage in the winter months. In the winter relaying in a category 'A' area for four weeks followed by depuration was needed to remove both *E.coli* and F+ phage.

2.6 Use of Physiological Assessment Techniques for Determining Relative Activity Rates of Bivalve Shellfish during Simulated Depuration

P. WILSON AND MARK R. BOULTER

Seafish Industry Authority, Seafish House, St. Andrews Dock, Hull, UK, HU3 4QE

ABSTRACT:
A series of trials were undertaken over an 18 month period to determine the relative activity rates for mussels *(Mytilus edulis),* Pacific oysters *(Crassostrea gigas),* native oysters *(Ostrea edulis),* Cockles *(Cerastoderma edule)* and Manila clams *(Tapes philippinarum),* subjected to varying seawater temperatures and dissolved oxygen levels. All these species are currently commercially depurated in the UK. To achieve this, alternative techniques to the more traditional use of bacteriological analysis were used to establish the physiological response of bivalve molluscs to varying conditions. These were the monitoring of ammonia excretion, consumption of dissolved oxygen and uptake of a neutral red dye. The monitoring of ammonia excretion correlated with dissolved oxygen consumption and these proved to be useful methods of obtaining information on the physiological response of bivalve molluscs subjected to varying simulated depuration conditions. The information obtained could not have been achieved by bacteriological analysis. However the dye test, although already an established method, did not prove to be entirely satisfactory. Overall, the results found that both species of oyster were much less active than the other bivalves, which may have implications for depuration systems. This work was funded by the Ministry of Agriculture, Fisheries and Food (MAFF).

KEYWORDS: shellfish, depuration, physiology, mussels, *Mytilus edulis,* Pacific oysters, *Crassostrea gigas,* Native oysters, *Ostrea edulis,* Cockles, *Cerastoderma edule,* Manila clams, *Tapes philippinarum*

UK SUMMARY:
The results show that by operating at a water temperature of 15 °C rather than the stipulated minimum temperatures, the activity of the shellfish would be at least double that at the stipulated minimum temperatures. There is concern about the generally low level of activity of both species of oysters and the associated facts that they are often eaten without cooking and are implicated in the majority of recorded incidences of food poisoning caused by bivalve molluscs in the UK. Their relatively low level of activity raises the question of whether a standard depuration period for all species, as currently practised, is appropriate or whether the depuration period for oysters should be longer, to provide a sufficient safety margin for these less active shellfish. Further microbiological studies, preferably viral, as most of the outbreaks are of viral origin, would be required to answer this question.

2.7 New Developments in Molluscan Shellfish in the United States

GARY E. RODRICK

Department of Food Science and Human Nutrition, University of Florida, Gainesville, Florida 3261 1, USA

Molluscan shellfish are filter feeding organisms that have the ability to concentrate both abiotic and biotic materials from their seawater environment into their body and tissues. Abiotic materials may include heavy metals, pesticides, herbicides and marine biotoxins. Biotic materials may include bacteria, viruses and parasites. If such shellfish are eaten raw and/or improperly cooked they can transmit disease, especially to the "at-risk" individuals suffering from liver disease or immune disorders. For these reasons, the molluscan shellfish industry is extensively regulated with HACCP with a primary focus on monitoring shellfish harvesting waters and time and temperature controls. Because of naturally occurring vibrio bacteria and sporadic and unpredictable blooms of red tide and the declining quality of the US shellfish harvesting waters interest has developed in the areas of harvest temperature matrix, processing including freezing, blanching and cold shock, irradiation and depuration. In summary, many changes are rapidly occurring in the US molluscan shellfish industry. Many of these changes coupled with educational programs will hopefully keep the molluscan shellfish industry stable and viable in the next few years.

UK SUMMARY:

HACCP will become mandatory under the Seafood Inspection Bill in the USA or for imports to the US from 15 December 1997. This includes training requirements for key function staff.

Vibrio's are the biggest shellfish problem in the US. Between 81 - 94 there have been 45 deaths and 86 hospitalisations from V.vulnificus. This is worst in people with liver disease, diabetes, alcoholism or gastro-intestinal problems. V.vulnificus is a naturally occurring marine organism that lives in low salinity warm water areas. The latest control mechanism is the adoption of time/temperature controls.

Temperature	Time to Refrigeration
<18°C	No limit
18-23°C	14 hours
23-28°C	12 hours
28°C+	6 hours

Other methods being studied are freezing, irradiation and the use of ozone.

2.8 Risk Assessment of *Vibrio vulnificus* Infection from New Zealand Oysters

DOROTHY-JEAN MCCOUBREY

Ministry of Agriculture, MAF Quality Management, P.O. Box 1254, Auckland, New Zealand

ABSTRACT:
Vibrio vulnificus (V. vulnificus) is a naturally occurring (autochthonous) pathogen and therefore cannot be controlled with the same methods that are suitable for sewage based problems e.g. *Salmonella typhi.* The bacteria affects only a small "at-risk" group - those with liver problems and those with iron storage diseases, but the health consequences for those in the "at-risk group" are severe. Persons with predisposition exposed to *V. vulnificus* can suffer septicemia with up to 70% mortality. Much is still not known about how or why *V. vulnificus* causes these severe health effects - the pathogenic dose and the infective strains are still to be identified. *V. vulnificus* thrives in water above 17°C and with salinity levels between 5-25 ppt. Such parameters occur in estuaries and consequently raw oysters seem to be the common shellfish agent. Cooking quickly destroys the bacteria. New Zealand undertook a study of the potential hazards associated with commercial shellfish operations causing *V. vulnificus* problems. This involved:

i) an environmental risk assessment and a sampling programme in commercial oyster growing areas;

ii) a survey of the stakeholders (bureaucrats, shellfish industry, at-risk persons, doctors and the general public) as to how they perceived the risk associated with V *vulnificus* and how these risks should be managed.

The conclusions from the study were:

i) The commercial oyster environment is not conducive to *V. vulnificus* becoming endemic. The limiting factor is salinity.

ii) There are statistically significant (>95%) different opinions amongst the various stakeholders. These opinion differences relate to risk perception and preferred risk management methods. 40% of the At-Risk group were prepared to expose themselves to the potential hazards of eating raw oysters.

KEYWORDS:*Vibrio vulnificus,* high mortality, environmental risk assessment, stake-holders perception

2.9 Incidence and Detection of Pathogenic *vibrio* sp. in a Northern New England Estuary, United States of America

STEPHEN JONES and BEATA SUMMER-BRASON

University of New Hampshire, Jackson Estuarine Laboratory, Durham, NH 03824, USA

ABSTRACT:

Many Vibrio species are capable of causing infections in humans. *Vibrio vulnificus and Vibrio parahaemolyticus* are part of the normal microflora of estuaries, and have been implicated in diseases from consumption of raw or uncooked shellfish. In the Great Bay Estuary of Maine and New Hampshire, oysters *(Crassostrea virginica)* and soft-shell clams *(Mya arenaria)* are harvested for commercial and recreational purposes. Only one incidence of V *parahaemolyticus* infection from shellfish consumption has been documented. Traditional methods and a gene probe assay were used to enumerate *V. vulnificus* in water from sites along salinity gradients from two tributaries to the main water body (Great Bay) of the estuary. *V. parahaemolyticus, Escherichia coli,* enterococci, faecal coliforms, *Clostridium perfringens,* nitrate, ammonium, orthophosphate, suspended solids, chlorophyll a, dissolved organic carbon (DOC), temperature and salinity were also measured. Results showed lower salinity and higher concentrations of dissolved nutrients, suspended solids, faecal indicator bacteria and chlorophyll a in tributaries compared to Great Bay. Both *Vibrio* sp. were detected more frequently and at higher concentrations in the tributaries. Multiple regression analysis suggested suspended solids was the most significant variable, accounting for 27% of the variance in *V. vulnificus* and V *parahaemolyticus* concentrations. However, the gene probe results showed DOC was the most significant variable for explaining (44%) the variance in *V. vulnificus* concentrations. The results suggest that improved detection methods can enhance the understanding of environmental conditions conducive to both growth and inhibition of these pathogens.

UK SUMMARY:

It was found that V.vulnificus peaks in the summer months and that relaying in a clean area for five days reduced the levels by 90-99%.

3. Papers on Toxic Algae

3.1 Shellfish Feeding Preferences: Implications for Public Safety and Management

SANDRA E. SHUMWAY

Natural Science Division, Southampton College Long Island University, Southampton, New York, 11968

ABSTRACT:
Blooms of toxic and harmful algae occur worldwide and their frequency and distribution are increasing. Filter feeding bivalve mollusc concentrate phycotoxins produced by these phytoplankton rendering them unfit for human consumption. It is the responsibility of public health organizations to monitor shellfish for toxicity and to certify them safe. Their task is often confounded by the fact that the rate and level of accumulation vary greatly not only between species, but between individuals of the same species at a given location. Further, accumulation is affected by both extrinsic and intrinsic factors. In some instances, these individual variations can be used to the advantage of regulatory agencies, saving their programs both time and money. Differences in toxin uptake, accumulation and depuration by various shellfish species will be discussed in the context of management and public health.

UK SUMMARY:
Bivalve shellfish feed on particles between 5 - 300 µm in size and experiments show that some cells are passed through the gut intact therefore selective digestion is occurring. Selective feeding trials were carried out using dinoflagellates (toxin producing algae). It was found that the native (European) oysters preferred them. Pecten (scallop) and Mytillus (mussel) species showed no signs of change in feeding behaviour. Pacific oysters (*Crassostrea gigas*), Hard shell clams (*Mercenaria mercenaria*) and Soft shell clams (*Mya arenaria*) all disliked dinoflagellates. In natural PSP blooms mussels were found to become toxic 2 weeks before surf clams (*Spisula solidissima*). However, mussels detoxified in a matter of weeks whereas the toxin maintained in the surf clams for years.

3.2 Effects of Temperature and Body Size on PSP Toxin Kinetics in Surfclams, *Spisula solidissima*

V. MONICA BRICELJ, A.D. CEMBELLA and D. LABY

Institute for Marine Biosciences, National Research Council, 1411 Oxford St., Halifax, N.S. B311 3Z1, Canada

ABSTRACT:
Surfclams, *Spisula solidissima*, support an important fishery in eastern North America. They attain a large size (shell length ~20 cm), detoxify relatively slowly, and are capable of enzymatic conversion (decarbamoylation) of paralytic shellfish poisoning (PSP) toxins. It is therefore of particular interest to examine the effects of temperature and body size on toxin kinetics in this species. Juvenile surfclams which attained the same initial toxicity [~3.2 x 10^4 ug STXeq (saxitoxin equivalents) 100g following laboratory exposure to high-toxicity dinoflagellate, *Alexandrium fundyense* (Isolate GtCA29), were detoxified for 2.4 months at 5, 12 and 21°C. The viscera detoxified significantly (85 to 96% toxin loss) in all treatments, and the detoxification rate was greater at 21°C than at lower temperatures. In contrast, non-visceral tissues increased in net toxicity during detoxification. This was attributed to the increase in concentrations (mol. g^{-1}) of the high-potency toxins STX and decarbamoyl saxitoxin (dcSTX), which indicates that production of these toxins via biotransformation exceeds their elimination rate. The increase in dcSTX concentration during detoxification was directly related to temperature, ranging from 3-fold at 5°C to 8-fold at 21°C. This suggests that the activity of the decarbamoylase responsible for the production of dcSTX (from STX), is temperature-dependent, and that toxin conversions can greatly influence detoxification kinetics. Juvenile clams (0.6 g wet tissue weight) accumulated and eliminated toxins significantly faster than adults (8.1 g). The difference in weight-specific toxin uptake rates could be attributed to the allometric relationship between body size and feeding rate. therefore, body size is an important variable to consider in PSP monitoring programs.

KEYWORDS: PSP toxins, detoxification, surfclams, temperature

3.3 Endocellular Bacteria of *Alexandrium tamarense, a* Causative Dinoflagellate of Paralytic Shellfish Poisoning

MASAAKI KODAMA and YUKIO NAGAHAMA

School of Fisheries Sciences, Kitasato University, Sanriku, Iwate, 022-01 Japan

ABSTRACT:
During the bloom of toxic dinoflagellates such as *Alexandrium tamarense,* shellfish become toxic by ingesting them. Human consumption of toxic shellfish causes severe poisoning called paralytic shellfish poisoning (PSP). Although there have been many works on PSP and its causative toxins, little is known about the biosynthetic pathway and biological meaning of toxins. Recently, we isolated bacteria from several species of toxic dinoflagellates and found that these bacteria produce PSP toxins though the toxin productivity is low. These facts suggest that PSP toxin-producing bacteria are involved in the toxicity of dinoflagellates. On the contrary, toxin production is confirmed in the axenic culture of dinoflagellates. Therefore, bacteria should be living in the cells of dinoflagellates, if they are involved in the dinoflagellate toxins. Previously, we demonstrated the occurrence of bacteria in the cells of *A. tamarense.* However, endocellular bacteria are rarely observed under microscope. Therefore, the occurrence of endocellular bacteria in toxic dinoflagellates is still not clear. In the present study, it is shown that the extract of the axenic culture of *A. tamarense* reacts with not only antibody against PSP toxin-producing bacteria, but also specific probe for 16s RRNA of PSP producing bacterium isolated from *A. tamarense* strain used in the experiment.

KEYWORDS: PSP, endocellular bacteria, *Alexandrium tamarense,* PSP-producing bacteria

3.4 Occurrence of PSP-Producing Bacteria in the Bivalve Tissue Which Becomes Toxic During the Bloom of *Alexandrium tamarense*

MASAAKI KODAMA and TATSUYA YURIMOTO

School of Fisheries Sciences, Kitasato University Sanriku, Iwate 022-01, Japan

ABSTRACT:
Bivalve become toxic during the bloom of toxic dinoflagellates such as *Alexandrium tamarense.* It is generally accepted that the amount of toxin accumulated in the bivalve is the difference between the toxin amount supplied from dinoflagellate and that released from the bivalve. In the field survey on the bivalve toxicity and the abundance of dinoflagellate, however, some phenomena which could not be explained by this mechanism have been pointed out; there is a lag time between the peaks of bivalve toxicity and abundance of dinoflagellate; bivalve toxicity often increases under the absence of dinoflagellate. On the other hand, we previously reported that PSP toxin-producing bacteria were isolated from several species of toxic dinoflagellates, and suggested that these bacteria are involved in the toxicity of dinoflagellates. In the present study, it is demonstrated that the digestive gland tissue of the bivalve which became toxic by ingestion of *A. tamarense* reacted with the antibody against PSP toxin-producing bacterium isolated from *A. tamarense.* Universal probe for I6S RRNA of eurobacteria also reacted with the tissue. These results indicate that PSP-producing bacteria incorporated into the bivalve with *A. tamarense* are living in the cells of the tissue for some period. These bacteria in the tissue seem to be involved also in the bivalve toxicity.

KEYWORDS: PSP, PSP toxin-producing bacteria, bivalve toxicity, *Alexandrium tamarense*

3.5 Investigations on the Initiating and Triggering Mechanisms Causing Harmful Algal Bloom

RICARDO P. BABARAN and R.A. ESPINOSA

Institute of Marine Fisheries and Oceanology, University of the Philippines in the Visayas, Miag-ao, Iloilo 5023 Philippines

ABSTRACT:
Algal blooms have been variably associated with environmental factors such as increasing temperature and excessive eutrophication. But the actual mechanisms that may be responsible for initiating and/or triggering such events have never been completely understood. In this paper, a theoretical formulation for the initiation and triggering of algal blooms is presented and discussed. This is then correlated with field observations of algal bloom events in Philippine waters. Results suggest a strong agreement between theory and field observations. These findings are highly significant because they will not only allow a better understanding of the life cycle of cyst-forming dinoflagellate like Pyrodinium bahamense var. compressum but will also provide the missing links that have long been hindering the prediction of algal events. Moreover, the new insights will hasten the formulation of more effective red tide management programs and ensure the safety of shellfishes for human consumption throughout the world.

KEYWORDS: harmful algal bloom, triggering mechanism, Philippines, prediction, red tide management, shellfish safety

3.6 Toxicity and Toxin Composition of Paralytic Shellfish Poison in Individual Shellfish at the Mouth of the Niko River, Hiroshima

SETSUKO SAKAMOTO and YUICHI KOTANI

Nansei National Fisheries Research Institute, Maruishi 2-17-5 Ohno-cho, Saeki-gun, Hiroshima 739-04, Japan

ABSTRACT:
Paralytic shellfish poisoning (PSP) toxins in the short-necked clam *Ruditapes philippinarum and* wild oyster *Crassostrea gigas* were analysed by HPLC to clarify any variations in PSP toxicity and toxin compositions in individual shellfish due to differences in growth, feeding conditions and species. Shellfish were collected at two stations, st. 1 and st. 2, at the mouth of the Niko River which flows into Kure Bay. St. 2 was located about 100m upriver from st.1. The toxicity range (MU/g fresh meat) in short-necked clams was from 3.0 to 13.8 at st. 1 and 0.35 to 12.0 at st. 2 The correlation between the toxicity and clam weight was not significant at st. 1, however, toxin content per individual increased with clam weight. On the contrary, toxicity was negatively correlated to clam weight at st. 2, and the toxin content per individual was low with no relation clam weight. These results suggest that clams are able to metabolize PSP toxins quickly with their growth. Major PSP toxins in the clams were Cl, C2, GTX I and GTX4. The toxin composition was almost constant among the clams. However, the toxin composition was different between clams and the oysters. The major toxins in both species were Cl and C2, but the proportion of GTX2+GTX3 was higher than that of GTX I +GTX4 in oysters. The major toxins from *A. tamarense* which was causative organism of PSP in Kure Bay were C2 and GTX4. These results suggest that the shellfish have specific pathways to metabolize PSP toxins.

KEYWORDS: paralytic shellfish poison, short-necked clam, oyster, *Alexandrium tamarense*

3.7 Shellfish Toxicity and Pyrodinium Cell Density in Bataan, Philippines (1994-1997)

RHODORA V. AZANZA, R.O. ROMAN and L. N. MIRANDA

ABSTRACT:
Cell density of *Pyrodinium bahamense var. compressum,* a Paralytic Shellfish Poisoning (PSP) causative organism and toxicity of *Perna viridis* off Bataan (Manila Bay, Philippines) during 1994 to 1997 were monitored based mainly on the samplings conducted by the Bataan Red Tide Testing Center. From January to September 1997, there was no *Pyrodinium* bloom and consequently, no shellfish toxicity. High *Pyrodinium* cell counts were observed during the months of May to September (1994-1996) and concentrations of cells were lowest during the colder months of November to February (1 994-1996). The toxicity of green mussels, *Perna viridis,* as determined by mouse bioassay, was higher during the months of *Pyrodinium* bloom.

KEYWORDS: shellfish toxicity, cell density, *Pyrodinium bahamense var. compressum*

UK SUMMARY:
There have been 783 confirmed cases and 42 deaths in Manila bay since1988 attributed to toxic shellfish. Pyrodinium bahamense var. compressum has caused problems in The Philippines, Malaysia, Brunai, Tanzania and Mexico. Green mussels have been found to be the most vulnerable species.

3.8 Accumulation and Biotransformation of Paralytic Shellfish Poisons (PSP) in Shellfish

M.L.C. YOUNG and D.K.O. CHAN

Department of Zoology, University of HongKong, Pokfulam Road, Hong Kong

ABSTRACT:
In Hong Kong, contamination of marine shellfish by paralytic shellfish poisons (PSP) displays a highly distinctive seasonal cycle, peeking in February-April when the seawater temperature warms up to 20-22°C. Shellfish from the "cleaner" oceanic waters on the southern-eastern parts of Hong Kong tend to show higher contamination levels. *Alexandrium catenella* was isolated from the seawater during one such toxic red tide in Hong Kong, and maintained an axenic culture. This species produces PSP and shows maximal PSP levels under conditions of 20-25°C, salinity of 35o/oo, and pH of 8-8.5. To study the uptake and release of PSP by shellfish, the green mussel *Perna viridis* was exposed to Alexandrium catenella for 15 days and then decontaminated by feeding with the non-toxic chrysophyte *Isochrysis galbana* for 25 days. PSP in algae and mussels were monitored by HPLC-FD. Individual saxitoxin analogues were quantified and shellfish toxicity calculated.. It was found that toxin accumulation in mussels reflected the toxin profile of the dinoflagellate which they ingested during the 15 day contamination period, with >80% of GTX6(B2), 10% GTX 1+4 and traces of CI+2 toxins. Mussel toxicity was found to increase with the duration of contamination, reaching a toxicity level of 700ug STX equivalent/100g tissue on day 15. The toxicity exhibited a two-step decline during the decontamination period, during which there was a shift of dominance from GTX6 to C toxins. The safety threshold level of 80ug/100 g was reached after 7 days of depuration. Thus *Perna viridis* exhibited a fast-uptake and release of PSP.

KEYWORDS: paralytic shellfish poison, accumulation, biotransformation

UK SUMMARY:
After 5 days of depuration toxin levels fell from 700 - 100 µg/100g. After a further 5 days they fell from 100 - 0 µg/100g.

3.9 Transmission of the Paralytic Shellfish Poisoning Toxins from Dinoflagellates to Gastropod

CHIH-YU CHEN[1] and HONG-NONG CHOU[2]

[1]*Department of Zoology, National Taiwan University, Taipei, Taiwan 10617, ROC*

[2]*Institute of Fisheries Science, National Taiwan University Taipei, Taiwan 10617, ROC*

ABSTRACT:
Purple clams, *Hiatula diphos*, are filter-feeding bivalves and maculated ivory shells, *Babylonia areolata* are carnivorous gastropods. Both shellfishes are popular seafood delicacies among the Taiwanese. *H. diphos* were forced to contain gonyautoxins in this research by feeding them with cells of *Alexandrium minutum*, a toxic dinoflagellate species responsible for the paralytic shellfish poisonings in Taiwan. The intoxicated purple clams of known toxicity and toxin composition were fed to *B. aureolata* to observe the transmission and transformation of gonyautoxins among these shellfishes. It was found that the toxin composition in bivalve and gastropod were similar to that in dinoflagellate. Our data provide evidence for food-chain transmission of PSP toxins, from dinoflagellate to gastropod through a filter-feeding bivalve. The transmitted gonyautoxins-1,-11,-III and -IV of *A. minutum* could only be found in the viscera of these shellfishes. There was a notable degradation of gonyautoxin-1 in the ivory shell that resulted in a decrease in toxicity while the total amount of toxins was accumulatively increasing.

KEYWORDS: dinoflagellate, filter - feeding bivalve, gastropod, gonyautoxins, food-chain transmission, *Alexandrium minutum*

3.10 Saxitoxin in Urine from Victims of Shellfish Poisoning

NINO ISMAEL PASTOR

Disaster Management Unit, Department of Health, 1640 Sulu corner San Lazaro Sts., Tayuman, Sta. Cruz, Manila, Philippines

ABSTRACT:
Between July 4, 1993 until July 12, 1993, eight patients from Orion, Bataan in the Philippines were hospitalized after a meal of mussels. Symptoms common to all victims were numbness, weakness, dizziness, floating sensation and drunken gait. Their mean age was 25 (range = 15-33) years. All were males. Urine specimens (24-hour volume) were obtained from the victims. The high performance liquid chromatography (HPLC) laboratory detected saxitoxin in 2 out of eight victim's urine specimens. Concentrations ranged from 154 to 371 micrograms. A diagnosis of Paralytic Shellfish Poisoning (PSP) was made. The six other patients without saxitoxin in their urine, were also probably cases of PSP. Factors favouring the recovery of saxitoxin in urine specimens were reviewed. Implications to the diagnosis and treatment of PSP were explored.

KEYWORDS: urine, saxitoxin, paralytic shellfish poisoning

3.11 Events of Paralytic Shellfish Poisonings in Hong Kong and the South China - Impacts, Speciation and Implications

KIN CHUNG HO

Environmental Studies Programme, Open University of Hong Kong, 30 Good Shepherd Street, Homantin, Kowloon, Hong Kong

ABSTRACT:
Since the early eighties, there has been an increasing trend of eutrophication and algal blooms (which some times happened in the form of red tides) in the waters of Hong Kong. Algal blooms were most severe in the early spring (March-May) and late autumn (October-November) of each year, with thousand tonnes of fishes died of deoxygenation. In association with algal bloom, monitoring results showed that there was a very steady concentration of Paralytical Shellfish Poisoning (PSP) toxins being accumulated in the locally collected shellfish samples. All PSP toxins varied from 300 MU.Kg-l to 1500 MU.Kg-1. While such concentration of PSP toxicity in local shellfishes is below the harmful standard of 2000 MU.KG-1 , this persisted amount of PSP toxins indicates a potential risk to human's injection of seafood. Most of the consumed shellfishes in Hong Kong are however imported. The origins of the marketed shellfish include the Guangdong Province of China, Philippines and New Zealand. The native shellfishes only occupy less than 20% of the market. The Health Department of Hong Kong Government announced that there were numerous reports of shellfish poisoning in the nineties. A similar trend of increases in ciguatoxic poisoning is discovered. These increases in seafood poisoning are in line with the increase in shellfishes import from the mainland of China. According to HPLC analyses, the major toxins persisted in the shellfish samples marketed in Hong Kong are: CTX's, STXI, STX2, GTX2, GTX3 and GTX4. The STX and CTX toxins usually comprise over 65% of the total PSP toxins. The GTX toxins comprise another 30% of the total concentration. It is noted that the composition of PSP toxins in shellfishes collected from Macau, Hong Kong and the Mirs Bay are very similar. The composition of PSP toxins in this group is quite different from that in the Daya Bay and eastern Guangdong group. The results suggested that PSP poisonings in Hong Kong are attributed to the shellfishes imported from the eastern coast of the South China Sea.

KEYWORDS:PSP toxins, shellfish poisoning in Hong Kong and the South China

3.12 PSP Monitoring in the Clam Aquaculture of Taiwan

CHIH-YU CHEN[1], TZONG-HUEI LEE[1], CHUNG-KUNG LU[2] and HONG-NONG CHOU[3]

[1] *Department of Zoology, National Taiwan University, Taipel, Taiwan 10617, ROC*

[2] *Institute of Oceanography, National Taiwan University, Taipei, Taiwan 10617, ROC*

[3] *Institute of Fishenes Sciences, National Taiwan University, Taipel, Taiwan 10617, ROC*

ABSTRACT:
Paralytic Shellfish Poisoning is the only algal-toxin-related seafood poisoning ever reported in Taiwan. It has been found that *Alexandrium minutum* (previously identified as *A. tamarense)* was to be responsible for the occasional incidents in the past ten years. A monitoring program for farmed shellfish has been implemented by Fishery authorities under the Council of Agriculture for six years. During the intensive monitoring period, only the cultured purple clams, including *Hiatula diphos and H. rostrata* in a few areas were found toxic. It was believed that the aquaculture methods and locations are factors involved in the initiation of toxic clams, since the specimens collected from the most of the wild areas were not toxic. Purple clam has been widely utilized and found to be good in removing the suspended solids and planktons in the eutrophic shrimp or fish ponds. The feeding nature makes the purple clams more susceptible to be intoxicated than other clams. Our monitoring program thus focused on the cultured purple clams and develop a routine for the fisherman to submit their products for toxin examination.

KEYWORDS: paralytic shellfish poisoning, purple clam, monitoring, *Alexandrium minutum*

3.13 Occurrence of Toxic Red Tides and Shellfish Poisons Inspection in China

LIANZHU WANG

Yellow Sea Fishery Research Institute, 106 Nanjing Road, Qingdao, PR China

ABSTRACT:
Industrial development and increase in aquaculture activities have caused ocean pollution through eutrophication that also sometimes have facilitated red tide occurrences. This paper deals with information on red tide occurrences in recent years. The major causative plankton organism and their harmfulness are included. It also introduces the management and inspection systems for shellfish poisons being used in China at present.

KEYWORDS: red tide, shellfish poison, test

3.14 Should Mollusc Toxicity be Considered of Public Concern in Mexico?: The Impact of *Gymnodinium catenatum, Pyrodinium bahamense var. Compressum and Pseudonitszchia australis* Blooms in the Pacific Coast of Mexico

JOSE LUIS OCHOAL [1], A.P. SIERRA-BELTRAN [1], G. OLAIZ-FEMANDEZ [2] and D. L. DEL VILLAR-PONCE [3]

[1]*The Center for Biological Research CIBNOR, Box 128, La Paz BCS 23000, Mexico*

[2]*Health Ministry, Health Environment Division, San Luis Potosi No. 191, Mexico City 06700, Mexico*

[3]*Ecology Ministry, SEMARNAP-PROFEPA, Perferico Sur 5000, Mexico City 04530, Mexico*

ABSTRACT:

The first world record of *Gymnodinium catenatum* as the main causative organism of a major toxic episode belongs to the Gulf of California, Mexico (Graham, 1943). A major recent study Cortes-Altamirano and Nunez-Pasten, 1996, confirms, the presence of PSP-like toxins in shellfish tissues in association to *G. catenatum* blooms in the same area. In contrast, at Acapulco and Salina Cruz harbors, the toxic episodes have been connected to blooms of *Pyrodinium bahamensis var. compressum.* The most dramatic effect of such episodes lies in the number of human victims, reaching official numbers higher than 500 hospitalized and over 20 deaths in the last 20 years. On the other hand, the diatom *Pseudonitzschia australis,* has been linked to large ecological loses in the Gulf of California. Recent ASP outbreaks have been associated to mass deaths of sea-birds, fish, dolphins, sea lions and whales (Sierra-Beltran et al, 1997). Together, the above marine microorganisms represent the main cause of concern for 90% of the toxic episodes that affect Mexico's Pacific coast.

3.15 Monitoring for Paralytic Shellfish Poisons in Scotland and the Outcome of Research to Replace the Use of Mice

SUSAN GALLACHER, F. MACKINTOSH, S. O'NEILL, I. RIDDOCH and F.G. HOWARD

FRS Marine Laboratory, P.O. Box 101, Victoria Road, Aberdeen, Scotland, UK

ABSTRACT:
In 1991, the existing monitoring programme for Paralytic Shellfish Poisons in molluscs, was expanded to take into account the requirements of EC Directive 91/492/EEC and associated UK legislation. This resulted in an increase in the number of samples processed by the AOAC mouse bioassay to quantify the levels of toxicity. However, the use of animals for such purposes is becoming increasingly unacceptable hence considerable effort has been made to evaluate alternative techniques. This presentation will describe some of the problems with PSP in Scotland and data generated from an evaluation of both an ELISA technique and a tissue culture based assay for use in a monitoring situation.

KEYWORDS: Paralytic Shellfish Poisons, ELISA, tissue culture, mouse bioassay

UK SUMMARY:

The Marine Lab have carried out comparative testing trials using saxitoxin with different PSP assessment methods. These found that the standard mouse test itself can have errors as high as 20%. A lab based Tissue Culture Based Assay (TCBA) correlated with the mouse test with 85 - 100 % confidence (100% at low levels). A field based TCBA, being developed by Jellett Biotek showed a 76% correlation. ELISA test showed 100% correlation at low levels but only 60% at > 80 µg/100g. This is also only based on STX and is not good for GTX 1 - 4 toxins.

The TCBA's show promise as a replacement for the mouse test.

3.16 The Mist [tm] Shippable Cell Bioassay Kits for PSP: An Alternative to the Mouse Bioassay

JOANNE F. JELLETT, LISA DOUCETTE and ELIZABETH BELLAND

Jellett Biotek Ltd. 1 0 1 Research Drive, Dartmouth, Nova Scotia, B2Y 3Z7 Canada

ABSTRACT:
A shippable cell bioassay kit called MIST™ (Maritime *In Vitro* Shellfish Test) has been developed by Jellett Biotek Ltd. for Paralytic Shellfish Poisoning (PSP). This technology is a cost-effective alternative to the mouse bioassay, while being 20 times more sensitive than the mouse. The kits provide pre-cultured cells and pre-mixed reagents, eliminating the need for tissue culture facilities or expertise, and are robust enough to be performed in the field or on a ship. They are available in three formats: fully quantitative, semi-quantitative and a qualitative (yes/no) version, called Mini-MIST™. The shelf life of the quantitative MIST ™ Kit, is three weeks, although this can be extended by freezing the cells to -100°C. The kits have been successfully shipped from Canada to as far away as Australia and New Zealand. The MIST™ technology has been used to determine total PSP toxicity in acid extracts of phytoplankton, lobster hepatopancreas, mussels, scallops, clams, cyanobacteria and processed food. It can be used to determine toxicity in phytoplankton samples as well. Results of parallel trials with the kits and the mouse bioassay will be summarized.

KEYWORDS: PSP, cytotoxicity, cell bioassay, alternative to mouse bioassay

UK SUMMARY:

An AOAC validation study is to be carried out for the kits in 1998.

3.17 Uptake and Detoxification Kinetics and Compartmentalization of DSP Toxins in Molluscan Shellfish

ALLAN D. CEMBELLA, A.G. BAUDER and M.A. QUILLIAM

Institute for Marine Biosciences, National Research Council, 1411 Oxford Street, Halifax, NS B3H 3Z1, Canada

ABSTRACT:
Diarrhetic shellfish poisoning (DSP) is a serious human ailment primarily with gastro-intestinal symptoms caused by the ingestion of shellfish contaminated by species of toxigenic dinoflagellates. This seafood safety problem is particularly acute in Asia and Europe, but the implications are global. Bivalve molluscs can acquire DSP toxins by suspension feeding upon toxic dinoflagellates from either the water column or from the benthos. Several previous studies have attempted to document the relationship between blooms of *Dinophysis* spp. and shellfish toxicity, but the role of toxic epibenthic species such as *Prorocentrum lima* is less well established. In the present study specific rates of DSP toxin uptake and biotransformation and detoxification were determined in the bay scallop *Argopecten irradians*, exposed to toxigenic *P. lima* cells in laboratory microcosms. Tissue and algal extracts were analysed by liquid-chromatography-mass spectrometry (LC-MS) for okadaic acid (OA), dinophysistoxin-1 (DTX1) and recently identified OA-esters. Metabolic conversion pathways of DSP toxins were examined *in vitro* by incubating purified DSP toxins with scallop tissue homogenates. The kinetics and metabolism of DSP toxins in shellfish must be reassessed, in view of recent advances in toxin analytical methods and new knowledge of *in vivo* biotransformations and specific toxicity of DSP toxins. Current and historical data on DSP toxicity among various shellfish species will be critically reviewed from this perspective.

KEYWORDS: Prorocentrum lima, Argopecten irradians, Dinophysis spp., toxic dinoflagellate, bay scallop, DSP, okadaic acid, toxin kinetics

UK SUMMARY:
A fed depuration trial was carried out. Depuration was seen to be in two phases in the visceral tissues. There was a rapid reduction to below the 2 µg/g action limit in two days, then a slower reduction of the remainder over 3 weeks. All other organs depurated in less than 2 days.

3.18 Uptake and Elimination of Neurotoxic Shellfish Poison (NSP) by Pacific Oysters *(Crassostrea gigas)* and Greenshell ™ Mussels *(Perna canaliculus)*

GRAHAM C. FLETCHER [1], BRENDA HAY [2] and MARGARET SCOTT [3]

[1] *New Zealand Institute for Crop & Food Research, Private Bag 92169, Auckland, New Zealand*

[2] *AquaBio Consultants Ltd., PO Box 560, Shortland St., Auckland, New Zealand*

[3] *New Zealand Institute for Crop & Food Research, Private Bag 11030, Palmerston North, New Zealand*

ABSTRACT:
Our research has shown that Pacific Oysters readily accumulate toxin when fed *Gymnodinium breve* cells. Depuration using either ozone or ultraviolet light as a water sanitizing agent at water temperatures between 15 and 20°C and at salinities between 24 and 33 °/oo, reduced NSP toxin concentrations from levels ranging up to 100 to around 20 mouse unit/100 g. Using a closed system and five day depuration time periods, minimum toxin levels were achieved within three days. In an attempt to determine why the oysters did not continue to detoxify after three days we determined which sites within the oyster contained the toxin. The digestive diverticula had the highest concentrations before and after detoxification. Toxins were also detectable in the gonads and in the labial palps before and after depuration, but the concentrations were insufficient to account for the failure to continue to detoxify beyond three days. Improving water quality (reduced ammonia and increased dissolved oxygen) by the use of an in-line biofilter had a positive effect on detoxification. Compared to oysters, New Zealand Greenshell ™ mussels did not concentrate significant algal toxins when fed *G. breve* cells in our system. The mussels were adversely affected both by being held in closed tanks, and by the presence of the toxic algae. The mussels responded by spawning, regardless of the season. We conclude that NSP levels in oysters can be reduced to safe levels by depuration in closed tanks while such a system would be unlikely to be suitable for mussels.

KEYWORDS: detoxification, neurotoxic shellfish poison (NSP), Pacific oyster *(Crassostrea gigas)*, Greenshell ™ mussel *(Perna canaliculus)*

UK SUMMARY:
In oysters reduction by depuration over 5 days was not complete, it only took levels down to about the 20 MU/100g action limit. It did not continue falling further. Toxin levels dropped in the gills but were constant in the gut.

4. Papers on Regulatory Controls

4.1 The Molluscan Shellfish Industry and Regulatory Authorities Working Together

BRIAN M. ROUGHAN[1], ALAN CAMPBELL[2] AND DON MITCHELL[3]

[1] *Ministry of Agriculture MAF, Private Bag, Blenheim, New Zealand*

[2] *Marlborough Public Health Services, P.O. Box 46, Bleinheim, New Zealand*

[3] *SQP and Sanford Mussel Factory, Havelock, New Zealand*

ABSTRACT:
This paper discusses how the New Zealand Shellfish Quality Assurance Programme, which is based on the USFDA programme, achieves compliance by involving the Shellfish Industry through out all stages of the programme. NZ's seafoods standards are developed nationally through a process under an organization called FIICC (Fishing Industry Inspection Certification Council). FIICC is a joint Industry and MAF Regulatory Authority organization that sets all the seafood safety and market access standards. Even at this national standards setting stage there is significant Industry input. Compliance with the standards though is achieved at a local level using cost centre delivery teams. Local cost centres are made up of Industry, Health and MAF representatives. The cost centres meet regularly to discuss and action requirements to meet the standards as well as implementing proactive initiatives beyond the requirements of the standards to enhance the shellfish quality assurance programme. This paper outlines how the Marlborough Shellfish Quality Programme (MSQP), the largest cost centre in New Zealand, coordinates the 19 Greenshell ™ Mussels *(Perna canaliculus)* growing areas in the Marlborough Sounds to ensure a safe product is harvested from the growing waters.

KEYWORDS: Cost centres, industry involvement, New Zealand

UK SUMMARY:
NZ exports 66,000 tonnes of greenshell mussels to 52 countries per year. This is worth NZ$100 million p.a.. To achieve this a partnership between industry and regulators is essential. Hygiene legislation is non -prescriptive, outcome based and applies HACCP. All standards are pre - agreed by industry through the FIICC, which is a legal entity made up of 10 industry and 2 MAF members.

There is a shellfish QAP managed by MAF. It is paid for entirely by industry including the cost of EHO's and MAF inspectors. The largest QAP centre, Marlborough Sound, costs NZ$800,000 p.a. to run, which equates to NZ$ 180 per hectare of shellfish growing area. The QAP centre monitors water microbiology levels, shellfish microbiology levels, biotoxin levels, rainfall, salinity and other parameters. It reports back pro-actively to industry and holds training workshops, as well as having input into future research requirements.

4.2 Status of Mollusc Sanitation and Management in China

YUPING MIAO AND QIAO QINGLIN

East China Sea Fisheries Research Institute, 300 Jun-Gong Road, Shanghai, 200080 China

ABSTRACT:
This paper gives an overview of molluscan shellfish fishery in China including shellfish production, sanitation and management. The distribution and pollution state of heavy metal, the organic chloride pesticide and PCB remained in the commercial shellfish collected from parts of the China coast are described. Shellfish biotoxins; bacteriological investigations are also presented in the paper. The assessment on the commercial shellfish qualities in several bays and estuaries are made based on the investigation. Management and regulations concerning public health and shellfish safety are also stated including monitoring of pollutants in shellfish, safeguarding environmental quality, hazard analysis of critical control points in shellfish product and carrying out the studies on shellfish depuration.

KEYWORDS: molluscan shellfish, sanitation, management

UK SUMMARY:
During the 1990's there has been a big increase in the number of toxic algal blooms occuring in Chinese waters.

Between 1993 and 1996 aquacultured scallop production has increased from 60,000 to 1,000,000 tonnes. In 1998 shellfish safety and quality regulations are to be issued including algal monitoring programmes. The regulations for frozen scallops will incorporate HACCP.

5. Conference Recommendations

The conference recommendations were brought together through three workshops run at the end of the conference. The workshops addressed :-

1. Molluscan shellfish safety : problems and needs in developing countries.
2. Enhancement of private sector/industry participation in molluscan shellfish safety programs.
3. Molluscan shellfish safety : research and development priority areas.

The recommendations of the three workshops are detailed below :-

Workshop 1: Molluscan Shellfish Safety: Problems and Needs in Developing Countries

Chair: *Prof. Gary Rodrick, University of Florida*
Co-Chair: *Prof. Stephen Jones, University of New Hampshire*
Rapporteur: *Dr. Nemesio E. Montaño, University of the Phillippines*

Identified Needs in Developing Countries:

1. Development of a directory of scientists and experts in all aspects of Molluscan Shellfish Safety.
2. Development of mechanism of exchange of information concerning Molluscan Shellfish Safety in the electronic (internet) and/or in the printed form (booklet).
3. Encouragement of collaboratory research/information and training exchange between and within regulatory, industry and research institutes.
4. Development of a Molluscan Shellfish Safety Manual that establishes regionally harmonized rules and regulations compatible or consistent with international standards.
5. Transfer of expertise on post-harvest technology and Molluscan Shellfish Sanitation Regulations to developing countries.
6. Transfer of information concerning the required regulations on the export of Molluscan Shellfish to developed countries and the development of a Molluscan Shellfish Sanitation Program to meet the requirements.
7. In all of these, where possible, the use of models such as the IOC-IEO Science and Communication Center, NSSP, ... etc.

WORKSHOP II : Enhancement of Private Sector/Industry Participation in Molluscan Shellfish Safety Programs

Chair: *Dr. Joanne Jellett*
Co-Chair: *Dr. Robert Bailey*

Problems and Recommendations

1. Coastal Estuary Management

Develop management plans through government and industry participation to come out with joint recommendations.

2. Education and Training

Given limited resources, education programs should be directed first to the industry and then to the general public. Access to information through networking e.g. IOC newsletter, etc. The internet is not thought to be the best route because industry is not 'wired' in everywhere.

3. Depuration/Purification

Research studies on viral contamination. Better coastal zone management to improve growing area (reclassification to higher category). Industry participation in decision-making related to clean up of polluted areas and new developments that would impact on the environment.

4. Industry and Government Co-operation

Setting up of local, national and international standards. Organisation of the shellfish industry. Involvement of key players of industry.

5. Cost

Cost-sharing between government and industry, taking into consideration industry size in both developed and developing countries, and activities which must remain under government funding such as recreational harvesting.

WORKSHOP 3 : Molluscan Shellfish Safety : Research and Development Priority Areas:

Chair : *Dr. Sandra Shumway*
Co-Chair : *Dr. Jorge Diogene*

Approximately 33 people attended the workshop on Research and Development Priority Areas on Molluscan Shellfish Safety. Participants agreed to list major issues in relation to research and development but without giving any ranking or priority to these issues. The subjects of discussion were grouped in the following sections: Bacteria and Viruses, Education, Mitigation and Treatment of Harmful Organisms, Other Issues.

Bacteria and Viruses:
As the need for a quick, cheap and easy detection methods for the presence of pathogens was raised, it was pointed out that scientists and managers should work "hand in hand" with the industry. *E coli* are not good indicators of human viruses. Appropriate indicator species would therefore be needed in order to cover a wide spectrum of pathogens. These should be used in parallel with direct detection and characterization of the pathogens themselves. These indicators (e.g. bacteriophages, enterovirus) should be appropriate to detect enteric viruses and be appropriate for extensive monitoring. Concern was raised in use of phages as indicator as they also occur in animals where human viruses are not found. This is relevant in areas where faecal input is animal not human. However work is being undertaken to differentiate phage from human or animal sources using gene probe, to see if this is a significant problem. A wider range of pathogenic agents (e.g. natural multiplying organisms in the environment) would also need attention, in particular regarding their pathogenicity and ecological significance. Depuration techniques would therefore need to address all pathogens. Epidemiology was also considered a major issue and the need to link more studies to monitoring and research was stated.

Unlike the viruses, *V. vulnificus* is not a worldwide problem. *V. vulnificus* is restricted to countries with warmer waters and is generally considered high priority. Other vibrio species such as *V. parahaemolyticus* and *V. cholerae* may be more of an issue.

Requirements exist to obtain data on the extent of viral contamination, in the environment and in the shellfish after harvesting, and any resultant human infection in order to establish an epidemiological basis to formulate risk assessments.

A better understanding of the fate and activities of problematic microorganisms within shellfish and harvest and post-harvest environments is needed, as well as species-specific depuration conditions that are commercially and biologically feasible, especially in areas that are highly contaminated with faecal pollution.

Education:
It was recognised that research and development depend on the availability of scientific data.

Therefore, the importance of the educational aspect including information distribution and training was stressed. The need to 'train trainers' was raised, and as an example in need to organise a workshop or course on sample collection for coastal/oceanographical studies was proposed. The need to inform physicians was also expressed. To respond to this issue it was stated that IOC, which has an active educational programme related with harmful algae, can support these course. However, the initiative from scientists and managers from the different areas is expected both in relation to the establishment of the training programme and funding raised from their governments and other institutions. The need for extensive training to form experts with mid-term/long-term exchange was pointed out.

Education should cover a wide spectrum of professionals and available information should reach the public.

Mitigation and Treatment of Harmful Organisms:
Low-technology strategies for mitigating sources of faecal contamination are necessary.

Regarding harmful algal blooms, several actions were presented in relation to mitigation strategies. It was stressed that discussion regarding their possible applications should be extremely cautious and environmental impact should be of major concern. It was suggested to better learn to "accommodate" nature and not alter it, especially in marine waters where water movement and transfer cannot be controlled. No large-scale introduction of any species or substance should be attempted until the consequences are well known.

Examples of mitigation techniques were:

- use of clay, with concern on the effect of sedimentation in benthos and eutrophication
- alteration of phytoplankton assemblages
- introduction of parasites (e.g. to alleviate *Alexandrium sp.*) with concern on the specificity that has to be clearly defined.

Other Research Issues:
Several other issues related with applied and fundamental research were raised. It was proposed that the effectiveness (UV)/ineffectiveness (Ozone) of methodologies for treatment for PSP toxins in depuration plants should be clearly stated. Information should be provided on the characteristics of toxin standards used worldwide in order to reach common agreement between methodologies. The need for more studies on the movement, biotransformation and biodegration of marine toxins through the food web was expressed. These studies could respond to the demand of better understanding the effects of toxins on target organisms, binding and mechanisms of action of toxins and the possible role of detoxifying enzymes. Stress was put on the development of treatments and antidotes. Toxicological studies and research on new toxins should also be favoured.

It was stressed that good scientific data are necessary to the establishment of regulations regarding shellfish safety.

International co-operation should be encouraged to focus on research and development activities in addition to training of personnel in developing countries.

6. Conclusions

There are three major food safety problems currently associated with the consumption of molluscan shellfish, viruses associated with sewage contamination, poisoning from naturally occuring toxin producing algae and the naturally occuring Vibrio species of bacteria.

The developing world has major problems in funding and carrying out monitoring for these problems. In the developed world monitoring programmes for these problems are in place. However, the funding of such monitoring programmes is often a point for much debate. There is also debate about whether the best testing methods are being used. The *E.coli* indicator organism is coming increasingly under fire as a good indicator of sewage pollution, due to the problem now being primarily one of viruses not bacteria. F+ bacteriophage is being suggested as an additional/alternative indicator organism for the presence of viruses. There have also been doubts raised about the MPN methodology of *E.coli* testing with call for it to be replaced by newer, less variable, methodologies. Ethical and consistency issues have also been raised about the use of mice for the toxic algal tests. Newer tissue culture based assays are currently being assessed and show some potential as a replacement method for some toxic algal determinations.

For viral pollution there is a need for more research into relaying and/or depuration techniques for the removal of viruses. There are signs that this work is likely to occur in the next few years. Equally for the toxic algae there is a need for research into relaying and /or depuration. With regard to Vibrio's we can count ourselves lucky that in the UK we do not have the correct conditions for *Vibrio vulnificus*, which causes many problems in USA. Where this problem does occur there is a need for continuing education of the "at risk" individuals and the continuation/development of effective controls such as time/temperature thresholds and freezing.

The industry should not be complacent even when operating within the current legal framework. Any of the above causative agents maybe lurking behind the next corner ready to disturb the status quo. It must be prepared to increase sampling regimes to include viruses and toxic algae in the future and be aware that short or longer term harvesting closures and/or changes to depuration systems and/or an increase in depuration times may become inevitable if the protection of the public and hence the survival of the industry is to be assured.

Appendix I

A Summary of the Illness Associated Organisms which can affect Molluscan Shellfish Safety

A Summary of the Illness Associated Organisms which can Affect Molluscan Shellfish Safety

1. Bivalve molluscs have the potential to cause disease from viral and bacterial microorganisms and from toxin producing algae . These agents are acquired from three sources:

   a) Faecal pollution of the aquatic environment
   b) The natural aquatic environment
   c) Post harvest contamination

A brief description of the main causative agents of molluscan associated illness and the testing and control measures routinely adopted to protect the public are outlined in the following summary.

2. Sewage Pollution Related Organisms

2.1 Bacterial Pathogens

Human illness caused by the consumption of molluscs contaminated due to seawater polluted with bacterial pathogens has largely been stopped due to a decrease in the incidence of bacterial illness, the adoption of depuration technology to purge shellfish grown in mildly polluted waters and the closing of heavily polluted harvesting areas.

The coliform indicator used to define water and product quality is an indicator of bacterial pathogens. The problem with the coliform indicator is that it does not correlate well with the presence of human enteric viruses, which are the pathogens now most commonly associated with sewage contamination of molluscs. Also it does not indicate the presence of non-sewage related naturally occurring bacterial pathogens such as *Vibrio*'s.

The EU currently use the US end product standard to define our clean water category rather than the US water standard. The US standards are:

A mean level of 14 *E. coli* MPN/100ML with <10% of samples exceeding 43 *E. coli* MPN/100ML and an end product standard of 230 *E. coli* MPN/100g of meat.

2.2 Viral Pathogens

The largest number of illnesses are reported from unknown etiologies clinically suggestive of Small Round Structured Viruses (SRSV's) or Norwalk and Norwalk-like agents of human enteric viral gastroenteritis. The vast majority of these cases are associated with the consumption of raw mollusc's taken from harvest waters contaminated with raw or poorly treated human sewage.

Classification of molluscan growing waters based on valid human enteric virus indicators, as well as proper treatment and disposal of sewage are required to deter raw shellfish associated viral infections. Currently there is no agreed indicator for human viruses in shellfish or growing waters.

Depuration and relaying remove enteric bacterial pathogens and indicators from mollusc's. However, the depuration of some enteric viruses may not proceed at the same rate. *Hepatitis A* virus (HAV) persists far longer in oysters and clams than *E. coli*. Depurated molluscs have been responsible for outbreaks due to enteric viruses.

3. Naturally Occurring Marine Microorganisms

3.1 Bacterial Pathogens

The main pathogenic, free living, natural bacteria are of the *Vibrio* species. These are not generally associated with faecal contamination and their numbers tend to be higher in the warmer summer months. *Vibrio*'s are responsible for fewer cases of illness than viruses. However, as well as causing gastroenteritis some species are associated with high mortalities in people who are immunocompromised or have liver disease. *Vibrio vulnificus*, which is common in waters warmer than 20°C, has, in the US, killed 45 people between 1981-1994.

Testing for *Vibrio*'s is not a problem and research into removal of *V.parahaemolyticus* and *V.vulnificus* by depuration and relaying is ongoing. Depuration is possible but not within the 42 hour period currently used for other bacterial removal.

3.2 Toxin Producing Algae

A number of species of mostly dinoflagellate algae found in plankton can produce toxins which cause illness in mammals. These toxins enter the marine food chain and can be concentrated by bivalve molluscs, such as mussels, scallops, oysters and cockles filter feeding on the algae. They do not normally cause a problem in other species of fish and shellfish in our waters.

Some of these toxins are harmful to humans but they do not usually affect the molluscs themselves. The toxins cannot be detected by taste. Shellfish can remain toxic for long periods after an algal bloom subsides. Each type of toxin poisoning is linked to specific algal species that produce different types of toxins. Each toxin type causes different illnesses.

Toxic algae are a global problem and throughout the world a number of different forms of poisoning have now been recognised :

PSP	Paralytic shellfish poisoning
DSP	Diarrhoeic shellfish poisoning
NSP	Neurotoxic shellfish poisoning
ASP	Amnesic shellfish poisoning

There are currently no commercial depuration methods for combatting toxic algal bioaccummulation. However, there is scope for research in this field. The standard control measure is to not harvest/catch the shellfish until levels of the toxin have subsided naturally. This requires regular routine monitoring to ensure that the algal toxins are not present.

3.2.1 PSP

Paralytic shellfish poisoning results from the consumption of molluscs that have bioaccummulated toxigenic dinoflagellates. The species of concern are *Pyrodimium bahamense var. Compressum*, *Alexandrium.tamarenses*, *Alexandrium minutum* and *Gymnodinium catenatum*. The cause of PSP is a complex of toxins known as saxitoxins which are neurotoxins. These are potentially life threatening however more usually cause tingling and numbness.

PSP can be tested for by Mouse bioassay, HPLC (high performance liquid chromatography), ELISA (Enzyme Linked Immunoabsorbant Assays) tests, though this method is not yet fully developed, and more recently developed mouse tissue culture based assays.

3.2.2 DSP

Diarrhetic shellfish poisoning is caused by *Dinophysis fortii, D.acuminata, D.mitra, D.caudata, D.miles* and *Procentrum lima*. A number of toxins have been identified most commonly Okadaic acid. Symptoms include diahorea, nausea and vomiting. This is not life threatening.

There is a mouse bioassay for DSP, HPLC can be used for Okadaic acid and other toxin determination.

3.2.3 NSP

Neurotoxic shellfish poisoning or brevetoxic poisoning is caused by the red tide organism *Gymnodinium breve*. This causes respiratory irritation/gastroenteritis and is not usually fatal.

There is a mouse bioassay for NSP.

3.2.4 ASP

Amnesic shellfish poisoning is the name given to the illness caused by consumption of domoic acid which is present in the diatom *Pseudonitzschia pungens* and *P.delicatissima.* The symptoms of domoic acid poisoning are vomiting, diarrhea, disorientation and memory loss. There have been reports of a few deaths in older people.

This can be tested for by HPLC.

4. Post Harvest Contamination

With live in-shell molluscan shellfish there is little risk of post harvest contamination as the shellfish are surrounded by their shell. They should, however, be kept away from possible sources of contamination such as dirty water, vessel bilges, seagulls, etc. The product should be kept in chill conditions at temperatures of <5°C. The main area of risk with in-shell product is if the shellfish are immersed in unsuitable conditions. Immersion should only take place in controlled conditions such as a purification plant.

Shucked molluscs should be handled in a hygienic manner and kept chilled to <5°C after processing to reduce the potential for bacterial growth.

Any cooking process which is used for molluscs should have control mechanisms to ensure that a 90°C core temperature is achieved for a minimum of 90 seconds to kill any viral organisms. Post cooking handling of the product should be kept to a minimum and temperatures should be held at below 5°C.